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Synthesis and Properties of Adenosyl- and Methylepicobalamin†

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ABSTRACT: Adenosyl-13-epicobalamin and methyl-13-epicobalamin have been prepared by reacting 13-epicob(I)alamin with 5′-*p*-toluenesulfonyladenosine and methyl iodide, respectively. The isomerization of the cobalamins at carbon-13 in trifluoroacetic acid is affected by the nature of the upper axial ligand. Treatment of methylcobalamin in trifluoroacetic acid does not yield the expected methyl-13-epicobalamin and reaction of 13-epicob(I)inamide with methyl iodide does not give an equimolar mixture of methylaquo-13-epicobinamide and aquomethyl-13-epicobinamide. The electronic spectra, circular dichroism, and optical rotatory dispersion of adenosyl-13-epicobalamin and methyl-13-epicobalamin are distinct from those of the cobalamins. Like the corresponding cobalamins, the two epicobalamins are converted to their "base off" forms in acid; however, the pK_a values for the 5,6-dimethylbenzimidazole moiety are lower (2.8 and 2.2) than those of the cobalamins (3.5 and 2.7). These lower pK_a values suggest that the inversion of the propionamide side chain at carbon-13 affects the electronic character of the cobalt atom. Adenosyl-13-epicobalamin does not function as a coenzyme in the ribonucleotide reductase system of *Lactobacillus leichmannii*, and acts as an inhibitor when incubated with adenosylcobalamin. Adenosyl-13-epicobalamin, methyl-13-epicobalamin, cyano-13-epicobalamin, and aquo-13-epicobalamin do not form active holoenzyme when incubated with N^5-methyltetrahydrofolate–homocysteine cobalamin methyltransferase apoenzyme from *Escherichia coli* B. However, these four epicobalamins were found to inhibit active holoenzyme formation with methylcobalamin.

When cyanocobalamin is treated with trifluoroacetic acid, or other highly acidic reagents, a mixture of cyanocobalamin, cyanocobinamide, and two darker colored corrinoids is formed (Bonnett *et al.*, 1971). Similar corrinoids are formed when corrinoid carboxylic acids such as cobyric acid are dissolved in strong acid. These new corrinoids are virtually indistinguishable from the corrinoids on the basis of electrophoretic behavior or ir spectra; while the electronic spectra show small but significant differences. On the other hand, their chiroptical and chromatographic properties are distinctly different.

The structure of these new corrinoids was elucidated by X-ray analysis. The X-ray data established that the propionamide side chain e attached to C-13 is projected up instead of down relative to the plane of the corrin ring. This inversion of configuration at C-13 causes a change in the conformation of ring C, while the rest of the molecule is not significantly altered (Stoeckli-Evans *et al.*, 1972). Scott *et al.* (1973) observed a downfield shift of one of the methyl groups at C-12 in the ^{13}C nuclear magnetic resonance spectrum (nmr) of dicyano-13-epicobinamide confirming the anticlinal relationship of this methyl group to the propionamide side chain at C-13. Hodgkin (Stoeckli-Evans *et al.*, 1972) has pointed out that in adenosylcobalamin the 5′-deoxyadenosyl moiety lies directly above C-13, a position which is occupied by the propionamide side chain in the 13-epicobalamins and thus the adenosyl moiety in adenosyl-13-epicobalamin must be positioned differently. On the other hand, the methyl moiety of methyl-13-epicobalamin would not be expected to be sterically hindered.

Because microbiological assays with *Escherichia coli* showed that cyano-13-epicobalamin is about 10% as active as cyanocobalamin (Bonnnett *et al.*, 1971) we decided to synthesize the two coenzyme forms of 13-epicobalamin and to determine their biological activity. This paper describes the synthesis of adenosyl-13-epicobalamin and methyl-13-epicobalamin. The physical and chemical properties as well as their coenzymatic activities are also reported.

Materials and Methods

Materials. Cyanocobalamin and DL-N^5-methyltetrahydrofolate were obtained from Sigma Chemical Co., DL-N^5-methyl-[^{14}C]tetrahydrofolate was from Amersham/Searle, *S*-adenosyl-L-methionine chloride was from P-L Biochemicals, 5′-*O*-(*p*-tolylsulfonyl)adenosine was from Zellstofffabrik Waldhof, and [^{13}C]methyl iodide, 61.8% enriched, was from Prochem. Ribonucleotide reductase from *Lactobacillus leichmannii* was kindly supplied by Dr. R. L. Blakley and N^5-

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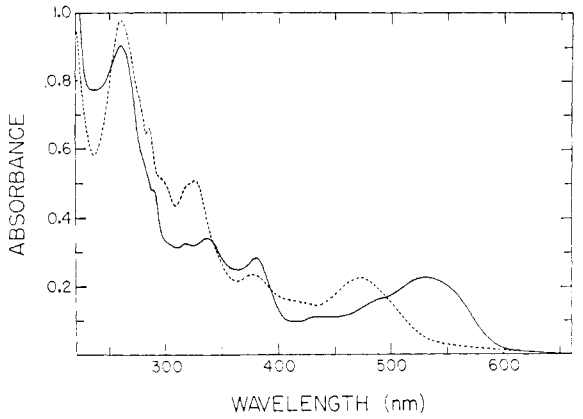

FIGURE 1: Adenosyl-13-epicobalamin, 0.03 mM; 0.1 M phosphate buffer (pH 7.0) (—), 0.1 N HCl (- - - - -).

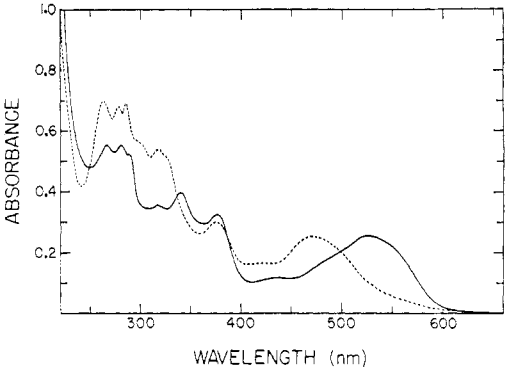

FIGURE 2: Methyl-13-epicobalamin, 0.03 mM; 0.1 M phosphate buffer (pH 7.0) (—), 0.1 N HCl (- - - - -).

methyltetrahydrofolate–homocysteine transmethylase apoenzyme from *Escherichia coli* was generously donated by Dr. R. T. Taylor. Thioredoxin and thioredoxin reductase were isolated from *E. coli* by the procedure of Laurent *et al.* (1964) and Moore *et al.* (1964) omitting the final gel filtration step. Adenosylcobalamin-5′-t_2 (22 Ci/mol) and adenosylcobalamin were prepared as described before (Gleason and Hogenkamp, 1971; Hogenkamp and Pailes, 1968).

Methods. Cyano-13-epicobalamin was prepared by incubating 1 g of cyanocobalamin in 20 ml of trifluoroacetic acid as described by Bonnett *et al.* (1971). After preparative paper chromatography on Whatman 3MM filter paper, cyano-13-epicobalamin was further purified by column chromatography on SP-C25 Sephadex (2 × 35 cm), to remove traces of cobinamides which were retained by this column. The desired epicobalamin was eluted with water, concentrated by the phenol–ether method and crystallized from aqueous acetone (Hogenkamp and Pailes, 1968), yield 100 mg. Adenosyl-13-epicobalamin and methyl-13-epicobalamin were prepared by reacting 13-epicob(I)alamin with 5′-*O*-tosyladenosine and methyl iodide, respectively, as described before for the preparation of the corresponding cobalamins. Both epicobalamins were crystallized from aqueous acetone. The purity of the epicorrinoids was established by paper chromatography in three solvent systems (Dolphin, 1971): solvent I, 2-butanol–water–ammonium hydroxide (50:36:14); solvent II, 1-butanol–ethanol–water (50:15:35); solvent III, 1-butanol–2-propanol–water (37:36:37) (Table I). Dicyano-13-epicobinamide, isolated by preparative paper chromatography (Bonnett *et al.*, 1971) was used without further purification for the preparation of the [^{13}C]methyl-13-epicobinamides.

Absorption spectra were recorded with a Cary Model 15 spectrophotometer. Circular dichroism (CD) and optical rotatory dispersion (ORD) spectra were recorded with a Cary Model 60 spectrophotometer. Concentrations of the 13-epicorrinoids were determined from the absorbance and the molar extinction coefficient of their α band. The molar extinction coefficients are based on ϵ_{367} 20.6 × 10^3 M^{-1} cm^{-1} (Bonnett *et al.*, 1971) for dicyano-13-epicobalamin. The epicobalamins were converted into dicyano-13-epicobalamin by photolysis in the presence of 0.1 M KCN.

Polarographic measurements were made using a Sargent Model XV polarograph as described before (Hogenkamp and Holmes, 1970). Ionization constants were determined by the spectral method of Ladd *et al.* (1961).

All radioactivity measurements were made with a Packard Model 3003 TriCarb liquid scintillation spectrometer using the naphthalene–dioxane counting fluid (Bray, 1960).

Assay Procedures. The ability of adenosyl-13-epicobalamin to function as a coenzyme in the ribonucleotide reductase reaction was determined by several different techniques. Ribonucleotide reductase activity was determined by measuring the amount of dATP formed from ATP by the diphenylamine method of Blakley (1966). Reductase activity was also measured spectrophotometrically with NADPH and the thioredoxin–thioredoxin reductase system of *E. coli* (Vitols *et al.*, 1967). Adenosyl-13-epicobalamin was also tested for its ability to replace adenosylcobalamin in the formation of the intermediate by the spectrophotometric stopped-flow procedure of Tamao and Blakley (1973) and for its ability to inhibit tritium exchange between adenosylcobalamin-5′-t_2 and water (Hogenkamp *et al.*, 1968). Furthermore, the enzymatic conversion of adenosyl-13-epicobalamin to 13-epicob(II)alamin and 5′-deoxyadenosine (Hamilton *et al.*, 1971) and the formation of the doublet signal (Hamilton *et al.*, 1972) were determined by electron spin resonance spectroscopy. The ability of the 13-epicobalamins to function as coenzymes in the N^5-methyltetrahydrofolate–homocysteine transmethylase reaction was determined by reaction 1 of Taylor and Weissbach (1967).

Kinetic constants were obtained from an unweighted nonlinear least-squares fit of the experimental data to equations for linear competitive and noncompetitive inhibition (Plowman, 1972) by means of a computer program provided by Dr. G. Gordon of Miami University, Oxford, Ohio. The enzyme kinetic subroutines were written by Dr. K. Sando of the University of Iowa.

TABLE I: Paper Chromatographic Properties of Some 13-Epicobalamins.

Corrinoid	$R_{cyanocobalamin}$ in Indicated Solvents		
	I	II	III
Cyano-13-epicobalamin	0.79	0.71	0.82
Methyl-13-epicobalamin	1.14	1.17	1.12
Adenosyl-13-epicobalamin	0.45	0.70	0.68

Results

Physical and Chemical Properties of the 13-Epicobalamins. The electronic spectra of adenosyl-13-epicobalamin and methyl-13-epicobalamin show small but definite differences from those of the corresponding cobalamins. Both epicobalamins differ from the cobalamins by the position of the broad maximum of the visible region (Figures 1 and 2 and Table II).

TABLE II: Absorption Spectra of 13-Epicobalamins.[a]

Epicobalamin	Position of the Main Absorption Bands
Adenosyl-13-epicobalamin	
0.1 M Phosphate buffer (pH 7.0)	260 288 335 379 527
	(29.5)(16.3)(11.7)(9.9)(7.8)
0.1 N HCl	260 284 327 378 470
	(32.2)(22.2)(17.1)(8.5)(8.0)
Methyl-13-epicobalamin	
0.1 M Phosphate buffer (pH 7.0)	265 280 289 318 340 378 438 528
	(17.0)(17.0)(16.0)(10.6)(11.9)(9.6)(3.2)(7.5)
0.1 N HCl	262 278 286 296 318 375 416 472
	(20.7)(19.9)(20.5)(17.5)(16.6)(8.5)(4.7)(7.6)
Aquo-13-epicobalamin	
0.1 N HCl	277 349 422 470 533
	(16.5)(16.4)(2.6)(2.9)(5.7)
Hydroxy-13-epicobalamin	
0.1 N NaOH	280 287 353 434 534
	(16.1)(14.9)(15.9)(3.8)(7.5)

[a] Position of main absorption bands is given in nm. Molar extinction coefficients ($\times 10^{-3}$) are given in parentheses.

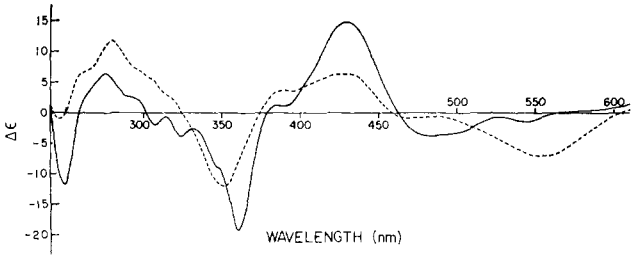

FIGURE 3: Circular dichroism of cyanocobalamin (—) and cyano-13-epicobalamin (- - - - - - - -) in 0.1 M phosphate buffer (pH 7.0).

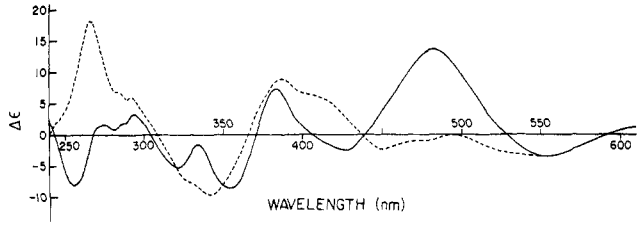

FIGURE 4: Circular dichroism of methylcobalamin (—) and methyl-13-epicobalamin (- - - - - -) in 0.1 M phosphate buffer (pH 7.0).

As in the case of the organocobalamins, addition of acid to solutions of adenosyl-13-epicobalamin and methyl-13-epicobalamin causes a color change from red to yellow indicating protonation and displacement of the coordinated dimethylbenzimidazole moiety. The apparent pK_a's estimated from the midpoint of the spectral changes are 2.8 and 2.2 for adenosyl-13-epicobalamin and methyl-13-epicobalamin, respectively. The CD spectra of cyano-13-epicobalamin, adenosyl-13-epicobalamin, and methyl-13-epicobalamin are shown in Figures 3–5. As pointed out by Bonnett *et al.* (1973) the CD spectra are typical for each series of cobalamins; the spectra of the epicobalamins are quite similar with intense positive maxima in the 260-nm region and broader maxima in the 350–450-nm region; the intense maximum at *ca.* 470 nm of the organocobalamins is completely lacking in the corresponding 13-epicobalamins. The CD spectra of the epicobalamins also show broad negative maxima in the region above 450 nm and more intense negative maxima in the 350-nm region.

The ORD spectra of the epicobalamins are distinct from those of the cobalamins, suggesting that the relatively small change at carbon-13 has a profound effect on the rotation (Table III).

The polarographic behavior of a few 13-epicobalamins is presented in Table IV, for comparison the half-wave potentials of the corresponding cobalamins are included. For all the corrinoids the first wave involves a two-electron reduction. The results indicate that only the half-wave potentials of cyanocobalamin and cyano-13-epicobalamin are significantly different.

Coenzyme Activity of the 13-Epicobalamins. Adenosyl-13-epicobalamin was unable to function as a coenzyme in the ribonucleotide reductase system of *L. leichmannii;* neither dATP production nor NADPH oxidation could be detected when this epicorrinoid replaced adenosylcobalamin. Adenosyl-13-epicobalamin did not undergo the rapid reversible spectrophotometric changes when incubated with equimolar quantities of reductase, dihydrolipoate, and dGTP. Furthermore, incubation of adenosyl-13-epicobalamin with excess ribonucleotide reductase, dihydrolipoate, and dATP did not yield the highly resolved esr spectrum; while incubation of the epicobalamin with the enzyme, glutathione, and dGTP did not produce the "doublet" esr spectrum. However, adenosyl-13-epicobalamin was able to inhibit the action of adenosylcobalamin in the ribonucleotide reductase reaction. The kinetic data were fit to equations for linear competitive and linear noncompetitive inhibition. Better fits were obtained for

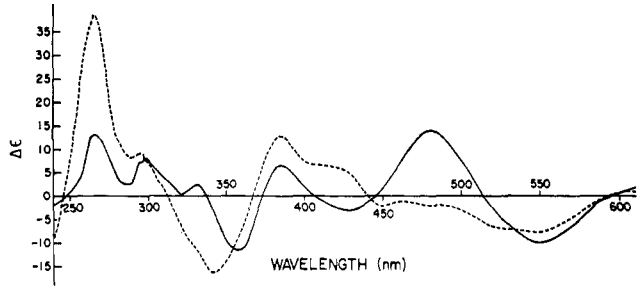

FIGURE 5: Circular dichroism of adenosylcobalamin (——) and adenosyl-13-epicobalamin (- - - - - - - -) in 0.1 M phosphate buffer (pH 7.0).

TABLE III: Optical Rotatory Dispersion of Some Cobalamins and 13-Epicobalamins.[a]

CN Cbl		CN epi Cbl		CH_3Cbl		CH_3 epi Cbl		Ado Cbl		Ado epi Cbl	
λ (nm)	[φ]	λ (nm)	[φ]	λ (nm)	[φ]	λ (nm)	[φ]	λ (nm)	[φ]	λ (nm)	[φ]
257tr	−47,500	252tr	−45,000	248pk	15,000	252tr	−46,250	250tr	−42,500	248tr	−90,000
		271i	−22,500	255	0					262	0
283	0	283	0	264tr	−22,500	267	0	278pk	−7,500	278pk	45,000
300pk	12,500			281i	−7,500	278pk	27,500	291tr	−17,500	293tr	22,500
310tr	2,500			293	0	286tr	22,500	305	0	311pk	33,750
320pk	12,500			304pk	15,000	305pk	35,000	331	0		
328tr	3,700	330pk	30,000	322	0			339pk	13,750	341	0
340pk	15,000			328tr	−7,500			353	0		
357	0	351	0	335	0	341	0	372tr	−36,250	367tr	−45,000
372tr	−62,500	372tr	−40,000	342pk	6,250			398pk	−2,500	394	0
403i	−40,000	399i	−12,500	348	0					412i	7,500
433	0	424	0	371tr	−38,750	368tr	−32,500			435pk	20,000
452pk	32,500	452pk	22,500	391	0	391	0	465tr	−27,500	465i	8,750
		487tr	12,500	396pk	2,500			482	0		
501	0	505pk	15,000	407	0			510pk	25,000		
557tr	−12,500	553	0	455tr	−28,750	431pk	23,750	550	0	530	0
		583tr	−15,000			470i	10,000	577tr	−20,000	577tr	−10,000
				481	0	477tr	7,500				
				514pk	3,500	510pk	13,750				
				572	0						

[a] pk = peak, tr = trough, i = inflection.

linear noncompetitive inhibition with K_{IS} values of 31.6 ± 4.2, 62.6 ± 11.8, and 37.9 ± 12.2 μM for dATP formation, NADP oxidation, and tritium exchange, respectively. Under identical assay conditions K_m values for adenoxylcobalamin were 5.1 ± 0.4, 3.1 ± 0.2, and 7.1 ± 1.2 μM, respectively. Several 13-epicobalamins were also tested for their ability to convert N^5-methyltetrahydrofolate–homocysteine transmethylase apoenzyme into an active holoenzyme. None of the 13-epicobalamins tested were able to produce an active holoenzyme; however, when these 13-epicobalamins were preincubated with apoenzyme, active holoenzyme formation with methylcobalamin was inhibited, suggesting that the 13-epicobalamins were able to interact with apoenzyme to yield a catalytically inactive complex (Table V).

Discussion

Although the chemical reduction of cyano-13-epicobalamin is more difficult than that of cyanocobalamin ($E_{1/2}$ = −1.24 V as compared with $E_{1/2}$ = −1.14 V *vs.* sce), the syntheses of adenosyl-13-epicobalamin and methyl-13-epicobalamin are analogous to the syntheses of the corresponding cobalamins.

The nature of the ligand in the upper coordination position affects the isomerization of the propionamide side chain at carbon-13. Incubation of methylcobalamin in trifluoroacetic acid as used for isomerization of cyanocobalamin does *not* yield the expected equilibrium mixture containing methylcobalamin, methyl-13-epicobalamin, methylcobinamide, and methyl-13-epicobinamide. Instead only methylcobalamin, methylcobinamide, and at most traces of the epicorrinoids could be detected. Furthermore, alkylation of epicob(I)inamide with methyl iodide does *not* yield nearly equal amounts of two stereoisomers: methylaquo-13-epicobinamide and aquomethyl-13-epicobinamide. The ^{13}C nmr spectrum of [^{13}C]methylepicobinamide shows only one major resonance

TABLE IV: Comparison of the Half-Wave Potentials of Cobalamins and 13-Epicobalamins.

Corrinoid	$E_{1/2}$ V *vs.* sce
Cyanocobalamin	−1.14
Cyano-13-epicobalamin	−1.24
Dicyanocobalamin	−1.32
Dicyano-13-epicobalamin	−1.34
Methylcobalamin	−1.38
Methyl-13-epicobalamin	−1.34
Adenosylcobalamin	−1.34
Adenosyl-13-epicobalamin	−1.35

TABLE V: Effect of Some 13-Epicobalamins on Holoenzyme Formation.[a]

Corrinoid	% Holoenzyme	% Inhibition
Methylcobalamin	100	0
Cyano-13-epicobalamin	0	54
Methyl-13-epicobalamin	0	86
Aquo-13-epicobalamin	0	81
Adenosyl-13-epicobalamin	0	70

[a] Mixtures (0.2 ml) containing apoenzyme (20 μg); the indicated cobalamins, 50 μM each; potassium phosphate (pH 7.4), 100 mM; β-mercaptoethanol, 0.2 M; *S*-adenosylmethionine, 50 μM; homocysteine, 250 μM; DL-N^5-methyl-[^{14}C]-H_4folate (2800 cpm/nmol), 150 μM; were incubated at 37° for 15 min in the dark. The reaction was stopped by adding 0.8 ml of ice–water.

at +0.17 ppm from tetramethylsilane, with only a trace of a second resonance further upfield at −3.31 ppm (approximate relative intensities 95:5). In contrast, the ^{13}C nmr spectrum of [^{13}C]methylcobinamide shows two approximately equal resonances (Needham *et al.*, 1973). The large difference in the intensities of the ^{13}C resonances of the two stereoisomers of the [^{13}C]methylepicobinamide complex suggests that one of the coordination sites is much less accessible to ligation. Furthermore, the observation that methylcobalamin does not equilibrate in acid to an equimolar mixture of epicorrinoids and corrinoids suggests that the methyl moiety sterically hinders the positioning of the propionamide side chain above the corrin ring. Alternately, the methyl moiety may bring about such changes in the electronic nature of the cobalt atom and the corrin ring that treatment with acid no longer yields the symmetrical intermediate at carbon-13.

The electronic spectra and the CD and ORD curves are distinct from those of the cobalamins. The spectrum of adenosyl-13-epicobalamin at neutral pH shows a broad maximum at 527 nm, which shifts to 470 nm in the "base off" form at pH 1; the corresponding maxima for adenosylcobalamin are at 522 and 458 nm. Similarly the maxima of the "base on" and "base off" forms of methyl-13-epicobalamins lie at higher wavelengths (528 and 472 nm *vs.* 519 and 462 nm). A comparison of the pK_a values of adenosyl-13-epicobalamin (pK_a = 2.8) and adenosylcobalamin (pK_a = 3.5) and of methyl-13-epicobalamin (pK_a = 2.2) and methylcobalamin (pK_a = 2.7) suggests that the inversion of the propionamide side chain at C-13 has a profound effect on the cobalt atom. The lower pK_a values of the epicobalamins indicate that the 5,6-dimethylbenzimidazole moiety is stronger coordinated to the cobalt atom suggesting that the cobalt atom of the epicobalamins is more electrophilic than the cobalt atom of the cobalamins. A change in the electronic character of the cobalt atom of the epicobalamin is also indicated by the ^{13}C nmr spectrum of methyl-13-epicobalamin. The chemical shifts of [^{13}C]methyl-13-epicobalamin in the "base on" and "base off" forms are +8.18 and +0.16 ppm, respectively, from Me_4Si while the chemical shifts of the corresponding forms of [^{13}C]methylcobalamin are +7.74 and 0.00 ppm from Me_4Si. Earlier studies have suggested that changes in the chemical shifts of the methyl ligand reflect changes in the electronic nature of the cobalt atom (Needham *et al.*, 1973).

The CD of adenosyl-13-epicobalamin, methyl-13-epicobalamin, and cyano-13-epicobalamin is typical of that of the epicorrinoids. However, compared to the CD of dicyano-13-epicobalamin (Bonnett *et al.*, 1971, 1973) the positive maximum at *ca.* 400 nm is less intense, while in the 250–300-nm region the former three epicobalamins show strong positive dichroism (Figures 3–5). The large differences between the CD curves of these epicobalamins and that of the more symmetrical dicyano-13-epicobalamin are undoubtedly due to the additional asymmetry caused by the difference in the axial ligands. As would be expected the ORD curves of the epicobalamins are different from those of the corresponding cobalamins (Table III).

Interaction of the Epicobalamins with Enzymes. Adenosyl-13-epicobalamin is not able to function as a coenzyme in the ribonucleotide reductase system of *L. leichmannii*. Furthermore, this analog of the coenzyme is not degraded by the enzyme as shown by the failure of the analog to give the highly resolved electron spin resonance spectrum of cob(II)alamin (epicob(II)alamin) when incubated with the enzyme, dihydrolipoate, and dGTP. In adenosyl-13-epicobalamin the adenosyl moiety is rotated away from its position occupied in adenosylcobalamin (Stoeckli-Evans *et al.*, 1972) and thus in the adenosyl-13-epicobalamin–ribonucleotide reductase complex the adenosyl moiety cannot be properly aligned in the active site. However, the observation that adenosyl-13-epicobalamin is not even degraded by the enzyme raises the possibility that more than just steric factors are involved. The low pK_a of the 5,6-dimethylbenzimidazole ligand of adenosyl-13-epicobalamin (2.8) indicates that the cobalt atom is more electrophilic. An increase in the electrophilicity of the cobalt atom would strengthen the carbon–cobalt bond and thus resist homolysis by the enzyme. In contrast, earlier studies (Yamada and Hogenkamp, 1972) have indicated that even drastic changes at the propionic acid side chain e, with the normal configuration at C-13, do not abolish coenzyme activity.

Electronic considerations may also explain the inactivity of the epicobalamins in the N^5-methyltetrahydrofolate–homocysteine transmethylase system. None of the four epicobalamins tested were able to form active holoenzyme when incubated with apoenzyme, all of them, however, did inhibit active holoenzyme formation from methylcobalamin and apoenzyme. X-Ray studies (Stoeckli-Evans *et al.*, 1972) have shown that the methyl group of methylepicobalamin is not sterically hindered by the propionamide side chain at carbon-13. However, the pK_a value of methylepicobalamin (2.2) as well as the ^{13}C nmr studies indicate that the electronic nature of the cobalt atom has been altered.

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The Chemical Synthesis and Nuclear Magnetic Resonance Spectroscopy of Adenosylcobalamin Selectively Enriched with Carbon-13†

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ABSTRACT: Adenosylcobalamin selectively enriched with 90% carbon-13 in the 5′-methylene carbon attached to cobalt has been synthesized from 90% enriched potassium [^{13}C]cyanide. The reaction sequence involves the condensation of labeled cyanide with L-erythrose to yield a mixture of L-[1-^{13}C]ribonic acid and L-[1-^{13}C]arabonic acid. L-[1-^{13}C]Ribonic acid is then inverted to D-[5-^{13}C]ribose by a series of reactions involving the oxidation of the 5-hydroxymethyl group to an aldehyde to become C-1 of D-ribose and reduction of C-1 of the acid to a hydroxymethyl group to become C-5 of D-ribose. The conversion of D-[5-^{13}C]ribose to [5′-^{13}C]adenosylcobalamin is then accomplished by published procedures. The proton and carbon-13 nuclear magnetic resonance spectra of [5′-^{13}C]adenosylcobalamin and its labeled precursors are also presented.

Adenosylcobalamin functions as a coenzyme in enzymatic reactions involving the transfer of hydrogen. For instance, in the dioldehydrase reaction a hydrogen from C-1 of propanediol is transferred *via* the 5′-methylene carbon of adenosylcobalamin to C-2 of propionaldehyde (Abeles, 1971). Similar hydrogen transfer has been shown with glyceroldehydrase, ethanolamine ammonia-lyase, and the adenosylcobalamin-dependent mutases (Barker, 1972; Stadtman, 1972). Ribonucleotide reductase also catalyzes such a transfer but in this reaction the hydrogen donor and acceptor are different compounds (Blakley, 1966).

Electron spin resonance (esr) data have indicated that during catalysis the carbon–cobalt bond of adenosylcobalamin is alternately opened and closed by a homolytic mechanism. Several authors have suggested that the 5′-deoxyadenosyl radical, formed in the initial homolytic fission, abstracts a hydrogen from the substrate to form 5′-deoxyadenosine and a substrate radical. The latter then combines with cob(II)alamin to form a new carbon–cobalt bond. Rearrangement of this substrate–cobalamin complex and cleavage of the carbon–cobalt bond yields cob(II)alamin and a product radical (Babior *et al.*, 1972; Cockle *et al.*, 1972; Finlay *et al.*, 1973). However, Blakley and coworkers (Tamao and Blakley, 1973; Orme-Johnson *et al.*, 1974) have pointed out that these paramagnetic species, also observed in the ribonucleotide reductase system, do not have the kinetic behavior expected of an intermediate in the reaction. Indeed, using stop-flow techniques with ribonucleotide reductase they were able to demonstrate the reversible formation of intermediates in the first 100 msec. The uv–visible spectrum of these rapidly formed species resembles that of cob(II)alamin, while the esr spectrum is quite distinct from that formed more slowly. The spin concentration is consistent with the production of two radicals, presumably a cob(II)alamin type paramagnetic species and a 5′-deoxyadenosyl radical.

In order to provide another technique for the study of the mechanism of the reaction, and more specifically to provide a probe for the adenosyl moiety, we have synthesized adenosylcobalamin enriched with ^{13}C in the 5′-methylene carbon. Recently carbon-13 enriched cobalamins and cobinamides have been prepared and analyzed by ^{13}C nuclear magnetic resonance (cmr) spectroscopy. These results have shown that cmr is a very sensitive technique for the study of the corrinoids (Needham *et al.*, 1973; Walker *et al.*, 1974).

This paper describes the chemical synthesis and the physical and chemical properties of [5′-^{13}C]adenosylcobalamin.

Experimental Section

Materials. Brucine, cyanocobalamin, and L-rhamnose were purchased from Sigma Chemical Co. Carbon-13 potassium cyanide, 90% enriched, was a gift from Dr. M. Goldblatt of LASL. Ribonucleotide reductase from *Lactobacillus leichmannii* was kindly supplied by Dr. R. L. Blakley. Aquocobalamin, 2,3-*O*-isopropylidene-L-rhamnose, L-erythrose, D-riburonic acid, and chloromercuri-*N*-benzoyladenine were

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