Circular Dichroism of some Vitamin B_{12} Derivatives [1]

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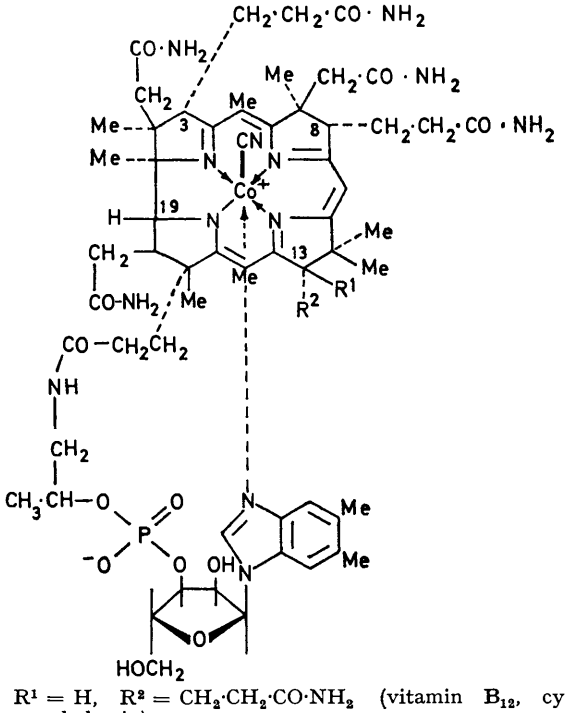
The c.d. spectra are recorded for a series of corrinoids possessing various structural and stereochemical features in the macrocyclic ligand. The solvent dependence of the dichroism of heptamethyl esters of dicyanocobyrinic acid and dicyano-13-epicobyrinic acid is reported. Contrary to a literature report, inversion of the c.d. at low temperatures is not observed.

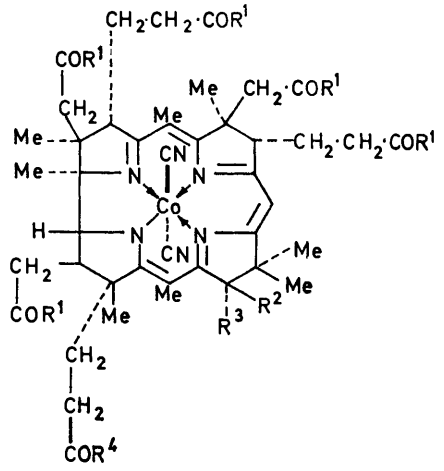
DURING work on the elucidation of the structure [2,3] of cyano-13-epicobalamin (neovitamin B_{12}) it was found desirable to carry out a survey of the c.d. spectra of vitamin B_{12} and its relatives. Particular attention was directed to those corrinoids in which variation in the macrocycle occurred: observations were made occasionally for solutions in water, but principally in 0·1M-potassium cyanide, since in this solvent the dicyanocorrinoids were formed, thus ensuring that differences in chiroptical behaviour did not arise from differences in the axial ligands.[4] Thus our study complements that of Williams and his colleagues, who examined [5] the



(I) R^1 = H, R^2 = $CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH_2$ (vitamin B_{12}, cyanocobalamin)
(II) R^1 = $CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH_2$, R^2 = H (neovitamin B_{12}, cyano-13-*epi*-cobalamin)

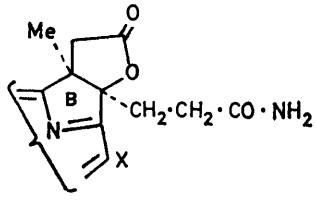
effect on chiroptical behaviour of variation of axial ligand. Some preliminary data recorded on various instruments were reported in outline earlier.[2] The present measurements, made on the Roussel–Jouan Dichrographe 185, are more accurate and more extensive.

Polyamides of the Normal Series.—This series comprised vitamin B_{12} (I), cobinamide (III), cobyric acid

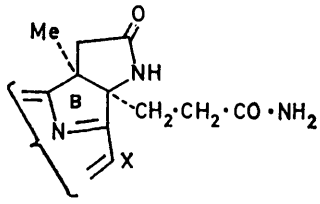


(III) R^1 = NH_2, R^2 = H, R^3 = $CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH_2$, R^4 = $NH{\cdot}CH_2{\cdot}CH(OH)Me$ (cobinamide)
(IV) R^1 = NH_2, R^2 = $CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH_2$, R^3 = H, R^4 = $NH{\cdot}CH_2CH(OH)Me$ (neocobinamide, 13-*epi*-cobinamide)
(V) R^1 = NH_2, R^2 = H, R^3 = $CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH_2$, R^4 = OH (cobyric acid)
(VI) R^1 = NH_2, R^2 = $CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH_2$, R^3 = H, R^4 = OH (neocobyric acid, 13-*epi*-cobyric acid)
(XI) R^1 = R^4 = OMe, R^2 = H, R^3 = $CH_2{\cdot}CH_2{\cdot}CO_2Me$ (heptamethyl cobyrinate)
(XII) R^1 = R^4 = OMe, R^2 = $CH_2{\cdot}CH_2{\cdot}CO_2Me$, R^3 = H (heptamethyl neocobyrinate, heptamethyl 13-*epi*-cobyrinate)

(all formulated as dicyanides)



(VII) X = H, vitamin B_{12} lactone
(IX) X = Cl, 10-chloro-derivative of the B_{12} lactone



(VIII) X = H, dehydrovitamin B_{12}
(X) X = Cl, 10-chloro-derivative of dehydrovitamin B_{12}

[Partial structure: remainder as in (I)]

(V), and a mixture of monopropionic acids designated [6] '1·6 m.' The c.d. of these compounds in 0·1M-potassium

[1] This paper is part of the Queen Mary College series on corrinoids. It is also Part 77 in the Westfield College Series on o.r.d. and c.d.; Part 76, C. C. J. Culvenor, D. H. G. Crout, W. Klyne, W. P. Mose, J. D. Renwick, and P. M. Scopes, *J. Chem. Soc.* (*C*), 1971, 3653.
[2] R. Bonnett, J. M. Godfrey, and V. B. Math, *J. Chem. Soc.* (*C*), 1971, 3736.
[3] H. Stoeckli-Evans, E. Edmond, and D. C. Hodgkin, *J.C.S. Perkin II*, 1972, 605.
[4] W. Friedrich, *Z. Naturforsch.*, 1966, **21***b*, 595.
[5] R. A. Firth, H. A. O. Hill, J. M. Pratt, R. J. P. Williams, and W. R. Jackson, *Biochemistry*, 1967, **6**, 2178.
[6] R. Bonnett, J. M. Godfrey, and D. G. Redman, *J. Chem. Soc.* (*C*), 1969, 1163.

cyanide showed similar features: details are collected in Table 1, and Figure 1 shows a typical spectrum. The

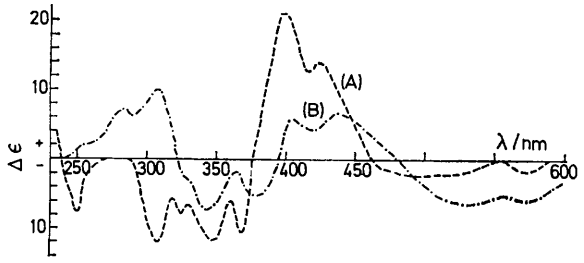


FIGURE 1 C.d. of vitamin B_{12} (A) and of neovitamin B_{12} (B) in 0·1M-KCN

spectrum obtained for dicyanocobalamin is very similar to that recorded earlier.[2,5] The spectrum was essentially unchanged over the concentration range 1·3—3·2 × 10^{-4}M, and at path lengths of 1 cm and 1 mm from 600 to 250 nm. However, close inspection of the experimental curves reveals that this range of concentrations is near the limit for meaningful measurements at 370 nm, which is the most intense absorption maximum in the isotropic absorption spectrum. Thus for a 1 cm path length there is only a weak inflection at 370 nm in the c.d. curve; with a 1 mm path length there is a clear negative maximum.

The preparation of cyano-13-epicobalamin (II) involves treatment of vitamin B_{12} with trifluoroacetic acid.[2] The c.d. spectrum of a crystalline sample of vitamin B_{12} recovered from this equilibration reaction was essentially identical with that of the authentic sample. This indicates that this recovered sample is not contaminated with appreciable amounts of other stereoisomers (*e.g.* compounds epimeric at C-3 and C-8) *provided* that separation from such isomers has not occurred during crystallisation. Thus we have no evidence for isomerisation, other than at C-13, during the treatment with trifluoroacetic acid, a finding which has been rationalised earlier.[2]

Polyamides of the 13-epi (*Neo*) *Series.* 13-Epimers [(II), (IV), and (VI), respectively] of cobalamin, cobinamide, and cobyric acid were examined together with the mixed monopropionic acids, designated[6] '1·3 dm' (13-epimers of '1·6 m'). The c.d. spectra (0·1M-KCN) were very similar throughout this series (Table 2) and distinct (Figure 1) from the spectra of the normal series. The main points of difference are as follows: (i) above *ca.* 480 nm a series of broad negative maxima occurs in each series: these are stronger for the neo-series: (ii) in the 400 nm region there are two strong positive maxima (at *ca.* 400 and 430 nm) in each series. These are at slightly shorter wavelength and are more intense for the normal compounds. A characteristic distinction is that the lower energy maximum is the less intense in the normal series: the reverse holds for the neo-series: (iii) in the region 300—370 nm the normal compounds show four strong negative maxima: the neo-compounds show two negative maxima at 340 and 380 nm, and a strong positive maximum at 308 nm: (iv) the normal compounds show a strong negative maximum at 250 nm.

TABLE 1

C.d. of some corrinoids of the normal series in aqueous 0·1M-KCN

λ/nm	Vitamin B_{12} (I) Δε	Cobinamide (III) Δε	Cobyric acid (V) Δε	1·6m Δε	Vitamin B_{12} recovered from equilibration with trifluoroacetic acid Δε
576—578	−1·56m	−1·82m	−2·70	−1·72m	−1·39m
528—531	−1·82sh	−2·04sh	−2·70 br,m	−2·41m	−1·67m
499—502	−2·34m	−2·27m	−2·70	−2·06sh	−1·95br,m
482—485	−2·08sh	−2·04sh	−2·00sh	−1·72sh	−1·39sh
423—425	+13·80m	+13·20m	+10·40m	+12·05m	+12·60m
395—398	+21·30m	+20·20m	+15·70m	+18·60m	+19·50m
364—368	−10·60m	−7·25m	−4·80m	−7·22m	−10·50m
344—348	−11·70m	−10·40m	−7·70m	−9·65m	+10·90m
323—325	−7·80m	−7·96m	−5·10m	−6·55m	−6·97m
306—308	−12·00m	−12·70m	−9·60m	−10·35m	−11·40m
297	−4·15sh				
279—280		−1·59m	−0·90m	−1·04m	
257—262	−2·07sh	−2·04sh			
249—250	−7·51m	−10·41m	−7·50m	−10·35m	−8·09m

m = Maximum, sh = shoulder, br,m = broad maximum.

TABLE 2

C.d. of some corrinoids of the neo (13-*epi*) series in aqueous 0·1M-KCN

λ/nm	Neovitamin B_{12} (II) Δε	13-*epi*-Cobinamide (IV) Δε	13-*epi*-Cobyric acid (VI) Δε	1·3dm Δε
576—578	−5·71	−5·96	−6·60	−6·17 m
531—533	−6·34 m	−6·51 m	−7·31 m	−6·49
505—509	−5·07	−4·61	−6·21	
435—437	+6·66m	+6·72m	+6·21m	+6·78m
404—405	+5·71m	+5·00m	+4·56m	+4·92m
378—379	−5·07m	−3·85m	−3·30m	−4·31m
340—343	−7·29m	−6·92m	−6·60m	−6·79m
328—333	−2·85sh	−4·22sh		−3·08sh
308—310	+10·20m	+10·00m	+10·22m	+10·80m
301			+8·80m	
280—283	+7·29m	+6·15m	+6·60m	+7·10m
236—237		−2·50m	−2·20m	−2·79m

There is no significant dichroism at this wavelength for the neo-series.

The spectra of four compounds were also measured in

water and in 2N-hydrochloric acid (Table 3). It is not certain what ligands are bonded to the cobinamides in these solvents but the observed differences between the vitamins and the cobinamides may be partly due to *co-ordinated* 5,6-dimethylbenzimidazole in the former.

TABLE 3

C.d. of some corrinoids of the normal and neo-series in water and in acid solution

Vitamin B_{12} (I)			
in H_2O		in 2N-HCl	
Δε	λ/nm	Δε	λ/nm
−2·25m	550		
−4·22sh	503	−3·37sh	517
−5·06m	486	−6·75m	490
+17·10m	432	+11·50m	433
+1·12sh	389	−5·06sh	388
−21·60m	363	−3·37sh	371
−10·40sh	348	+3·93m	354
−4·78m	326	−6·18m	320
−2·81m	308	−5·90m	303
+6·47m	276		
−14·10m	249	−14·30m	253
Neovitamin B_{12} (II)			
in H_2O		in 2N-HCl	
−9·38m	557	−7·26m	549
−1·81sh	477	−2·12m	482
+7·87m	432	+9·08m	422
+4·54sh	396	+1·21m	390
−14·50m	353	−12·10m	353
+12·70m	280	−9·99m	281
+6·96sh	265		
−2·12m	249	−2·12m	247
Cobinamide (III)			
in H_2O		in 2N-HCl	
+2·25m	554	+2·25m	559
−6·08m	490	−7·21m	492
+14·90m	433	+14·20m	436
−2·03m	385	−3·85m	388
−2·48m	370	−1·57sh	372
+3·61m	353	+5·41m	351
−7·44m	319	−7·21m	318
−6·99m	305	−6·08m	304
−13·1m	252	−12·2m	252
13-*epi*-Cobinamide (IV)			
in H_2O		in 2N-HCl	
−6·50m	532	−6·26m	540
+8·89m	429	+10·10m	424
−1·45sh	384	−0·96m	388
−6·50m	348	−7·71m	352
−4·34sh	326	−5·30m	323
+9·64m	281	+9·88m	280
		−1·20	251

The spectra of the vitamin and neovitamin are in some ways more similar to one another than are those of the dicyanides: it may be that replacement of the bulky 5,6-dimethylbenzimidazole ligand by a cyanide ligand allows any deformation of the chromophore resulting from the epimeric change to show up more readily.

Equilibration of Cobyric Acid (V) *and* 13-epi-*cobyric Acid* (VI).—In earlier studies the equilibration of the two series of corrinoid polyamides has been followed by paper chromatography. We have now examined the equilibration using c.d. Treatment of cobyric acid and of 13-*epi*-cobyric acid with trifluoroacetic acid at 25° for 17 h, followed by removal of the solvent *in vacuo* gave red residues, which, when dissolved in 0·1M-potassium cyanide gave purple solutions which showed very similar dichroism, possessing characteristics intermediate between those already outlined for the two series (Table 4). Thus the negative maxima above 480 nm were prominent, and a negative maximum was evident at 250 nm. On the other hand the maximum at 403 nm was more intense than that at 431 nm. A comparison of the ratio of dichroism at 400 and 430 nm gives for cobyric acid 1·64; for 13-*epi*-cobyric acid 0·65; and for the equilibrated mixtures from these two acids 1·18 and 1·16, respectively. It follows that the equilibrium mixture contains about 52% of cobyric acid. This is the first semiquantitative estimate of the composition of the equilibrium mixture: it agrees with

TABLE 4

C.d. of samples of cobyric and 13-*epi*-cobyric acids after equilibration with trifluoroacetic acid

	Cobyric acid	13-*epi*-Cobyric acid
λ/nm	Δε	Δε
576—577	−4·95 } m	−5·01 } m
532—533	−5·09 } m	−5·28 } m
510	−3·96 } m	−4·35 } m
431	+7·20m	+7·30m
402—403	+8·90m	+8·50m
367—371	−2·98m	−2·06m
241—242	−6·94m	−6·63m
326—329	−3·23sh	−2·75sh
311—313	+2·74m	+2·52m
299	+2·85m	+2·52m
280—285	+3·84m	+3·45m

earlier estimates based on visual examination of chromatograms.[7]

Fused Lactam and Lactone Rings at Ring B.—The c.d. of the vitamin B_{12} lactone (VII), dehydrovitamin B_{12} (VIII), and the hexa- and penta-carboxylic acids[8] (in 0·1M-KCN) are summarised in Table 5. Although there are some important differences (*e.g.* the weak positive maximum at 528 nm), the spectrum of the lactone shows many features in common with that of vitamin B_{12}: the other three compounds form a distinct group with many similarities. The maxima at 418—420 and 392—395 nm are slightly shifted to the blue with respect to the parent vitamin, and all four show a negative maximum at 277 nm which is not found in the B_{12} lactone or vitamin B_{12} itself.

Electrophoresis experiments have shown that the lactone, and not the lactam, opens in 0·1M-potassium cyanide, and this may well account for the differences between the lactone and lactam spectra in this solvent. The 10-chloro-derivatives [(IX) and (X), respectively] of the lactone and lactam show spectra similar to those of the two parents, but in each case many of the peaks are shifted to the blue (Table 5). The similarity is surprising since the introduction of the chloro-substituent at position 10 would be expected to cause overcrowding and perhaps deform the chromophore.

[7] R. Bonnett, J. M. Godfrey, V. B. Math, E. Edmond, H. Evans, and O. J. R. Hodder, *Nature*, 1971, **229**, 473.

[8] R. Bonnett, J. R. Cannon, A. W. Johnson, and Sir Alexander Todd, *J. Chem. Soc.*, 1957, 1148.

TABLE 5

C.d. of some lactones and lactams related to vitamin B_{12} in aqueous 0·1M-KCN

λ/nm	B_{12} lactone (VII) Δε	Dehydrovitamin B_{12} (VIII) Δε	Hexacarboxylic acid Δε	Pentacarboxylic acid Δε	Chloro-B_{12} lactone (IX) Δε	Chlorodehydro-B_{12} (X) Δε
594	+2·16m					
576—581		−0·93m	−3·72m	−3·28m		
555	+1·80m					
533—548		−0·93m	−3·16m	−2·69m		
490	−2·16m					
475	−1·80m					
			+1·30sh (470 nm)	−1·74sh (460 nm)		
418—426	+11·90m	+8·96m	+7·44sh	+6·56sh	+9·96sh	+9·09sh
394—398	+18·70m	+12·70m	+11·00m	+11·60m	+19·90m	+16·20m
368—373	−7·92m		+6·51sh	+6·56sh	−3·65sh	
343—346	−10·40m	−7·73m	−5·58m	−5·40m	−9·63m (353 nm)	−6·06m (351 nm)
322			+1·67m	+0·58m		
304—312	−10·40m	−4·64m	−5·12m	−5·21sh	−8·96m	
301—303			−5·77m	−5·79m		−5·02m
276—278		−6·49m	−8·56m	−8·68m		−4·04m (265 nm)
250—254	−7·92m	−4·33m	−4·65m	−4·63m	−11·30m	−6·06m
212—219	+14·40m	+23·20m	+24·20m	+22·20m	+6·64m (225 nm)	+17·20m (220 nm)

TABLE 6

C.d. of heptamethyl dicyanocobyrinate (XI) in solvents of differing polarity

10% EtOH–H_2O–CN^-		EtOH–HCN		$CHCl_3$–HCN		Iso-octane–HCN	
Δε	λ/nm	Δε	λ/nm	Δε	λ/nm	Δε	λ/nm
−3·54m	581	−5·38m	584	−8·60m	587	−7·76m	589
−3·07m	536	−3·03m	539	−4·85m	546	−4·43m	547
−2·83m	500	−2·48m	505	−3·05m	511	−2·77m	510
		−1·93sh	484	−1·62sh	408		
+13·00m	426	+13·50m	426	+12·80m	429	+14·10m	429
+20·80m	398	+22·70m	397	+23·00m	397	+25·80m	396
−7·09m	368	−9·10m	367	−8·10m	368	−9·97m	368
−10·90m	347	−12·70m	348	−12·80m	350	−12·70m	348
−7·56m	326	−6·89sh	329	−6·50sh	330	−6·65sh	330
−11·30m	308	−9·37m	310	−7·00m	311	−7·48m	311
		−3·86sh	298	−2·70sh	299	−3·60sh	301
		+1·93sh	285	+2·16sh	287		
		+2·21m	277	+5·21m	279	+3·60m	278
−12·00m	252	−12·70m	253	−11·70m	255	−13·80m	254
−9·21sh	244	−10·80sh	247	−9·70sh	248	−12·70sh	247
+10·60m	218	+22·10m	219			+19·40m	222
−16·50!	198	−35·90!	203				

! = Lowest wavelength measured.

Heptamethyl Esters.—The hepta-alkyl esters of the corrinoids, in contrast to the corrinoid polyamides, are soluble in a wide variety of organic solvents, and we have studied the effect of decreasing solvent polarity (aqueous ethanol, ethanol, chloroform, iso-octane), again using the dicyanide system, and maintaining it by incorporating a trace of hydrogen cyanide in the solvent. As shown in Table 6 a red shift is observed in the c.d. of heptamethyl dicyanocobyrinate (XI) as the polarity of the solvent is decreased. The effect is small (2—3 nm) for the higher energy transitions, but shifts of about 10 nm are observed for the bands which occur in aqueous ethanol at 500, 536, and 581 nm on moving to chloroform as solvent (Figure 2). There are additional changes in relative intensity: thus in solvents of lower polarity the negative maximum at 580 nm increases, while that at *ca.* 325 nm becomes a shoulder. Similar effects (red shift, minor intensity changes) are observed (Table 7) with heptamethyl dicyano-13-*epi*-cobyrinate (XII).

The c.d. spectra of the individual epimeric esters follow the general patterns observed with the two polyamide series (Figure 2). An attempt to observe an interconversion between these epimeric esters by c.d.

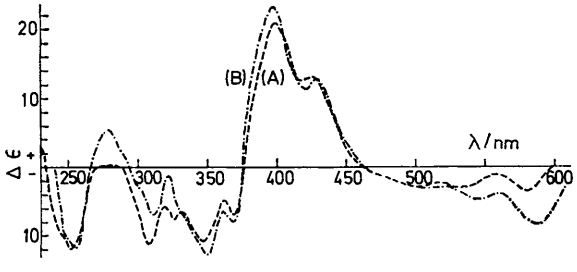


FIGURE 2 C.d. of heptamethyl dicyanocobyrinate in 0·1M-KCN–aqueous ethanol (A) and in HCN–chloroform (B)

following treatment of the individual esters with trifluoroacetic acid (18°; 24 h), indicated that little or no equilibration had occurred under these conditions. This observation supports the earlier suggestion [2] of the

importance of *amide* protonation in determining the course of this reaction.

TABLE 7

C.d. of heptamethyl dicyano-13-*epi*-cobyrinate (XII) in solvents of differing polarity

10% EtOH–H_2O–CN^-		$CHCl_3$–HCN	
Δε	λ/nm	Δε	λ/nm
−6·27 } m	582	−7·75 } m	591
−6·27 } m	533	−6·09 } m	535—345
−5·48 } m	509	−6·46 } m	512
+6·53m	439	+7·01m	440
+4·70m	408	+4·45m	409
−4·70m	379	−5·72m	378
−6·00m	342	+2·12m	364
−2·55sh	329	−3·14m	344
+11·20m	311	+10·20m	316
+7·83m	281	+9·60m	281
+3·92sh	268	+3·32sh	262
−1·57m	236	−1·29m	241

Effect of Temperature on Circular Dichroism of Vitamin B_{12} and its Derivatives.—Williams and his colleagues have reported [5] a remarkable dependence of the c.d. of vitamin B_{12} on temperature, which resulted in the curves at room temperature and −180° having almost a mirror-image relationship. We have not been able to observe this effect. Using the same solvent system (methanol–ethanol, 1 : 4) as the earlier workers we have found a gradual change, but no inversion of the curve, at temperatures down to −180° (Figure 3 and Table 8). Occasionally at −180° the record was erratic, and this we attribute to changes (*e.g.* developing opalescence due to partial crystallisation) in the sample.

Since the inversion effect would represent a formidable complication in the assessment of c.d. in the corrinoid series, we have attempted to observe it with other systems, but again without success. The c.d. of cyano-13-*epi*-cobalamin (II) in the methanol–ethanol solvent,

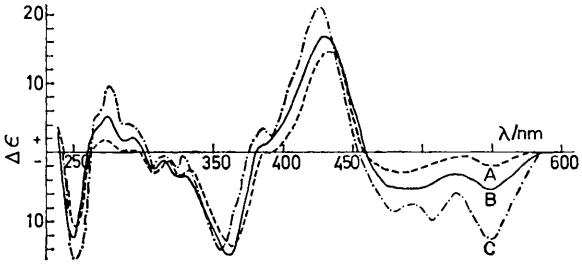


FIGURE 3 C.d. of vitamin B_{12} in methanol–ethanol (1 : 4) at −10° (A), −80° (B), and −180° (C)

and of heptamethyl dicyanocobyrinate (XI) in the less polar ether–isopentane–ethanol solvent mixture (Table 8), in each case changes gradually as the temperature is lowered, and no inversion of the curve is observed. We are, at present, unable to reproduce, or to account for, the earlier result.

TABLE 8

Low temperature c.d. of vitamin B_{12} and of heptamethyl dicyanocobyrinate

λ/nm	Δε (−10°)	Δε (−40°)	Δε (−80°)	Δε (−120°)	Δε (−160°)	Δε (−180°)
Vitamin B_{12} in methanol–ethanol (1 : 4)						
547—553	−2·13m	−3·63m	−5·46m	−7·35m	−10·50m	−12·70m
500—507	−2·35sh	−3·36sh	−4·96sh	−6·40sh	−8·68m	−9·04m
478—483	−2·98m	−3·63m	−5·21m	−6·40m	−8·23m	−8·59m
427—432	+14·50m	+16·10m	+16·90m	+18·00m	+20·1m	+20·8m
383—387		+1·29sh	+0·99sh	+1·42sh	+2·74m	+3·16m
355—363	−13·50m	−13·50m	−14·90m	−13·70m	−14·20m	−14·00m
323—327	−3·40m	−2·85sh	−3·47m	−2·83sh	−2·74m	−2·26m
306—308	−2·98m	−1·81m	−2·48m	−1·90m	−1·83m	−2·26m
290—294		+1·81m	+1·98m	+3·08m	+3·66m	+4·07m
274—275	+1·71m	+3·88m	+5·21m	+7·58m	+10·30m	+9·49m
248—250	−10·70m	−10·40m	−12·49m	−12·30m	−14·60m	−15·40m
Heptamethyl dicyanocobyrinate in ether–isopentane–ethanol (5 : 5 : 2)						
583—588	−3·93m	−3·58m	−4·18m	−6·15m	−6·73m	−7·18m
572	−2·28sh					
532—543	−2·07sh	−2·19sh	−2·09sh	−2·17sh	−1·95sh	−2·47sh
525	−2·48sh					
486—490	−4·97m	−4·78m	−4·75m	−4·71m	−4·78m	−4·90m
417—429	+11·6m	+11·9m	+12·7m	+13·5m	+14·2m	+14·2m
397—399	+15·3m	+15·9m	+16·9m	+16·3m	+17·4m	+17·5m
366—386	−6·42m	−6·77m	−7·22m	−7·24m	−6·90m	−6·83m
344—346	−6·65m	−7·16m	−7·41m	−7·60m	−8·32m	−8·57m
325—327	−3·93m	−4·38m	−4·75m	−5·79m	−6·73m	−7·00m
308—309	−6·42m	−6·77m	−6·84m	−6·52m	−7·08m	−7·18m
299—300		−4·18sh	−3·99sh	−2·90sh		−2·80sh
283—284	+1·35sh		+1·90sh	+3·08sh	+3·36sh	+3·22sh
275—277	+1·66m	+2·19m	+3·23m	+4·71m	+5·31m	+5·95m
252—254	−11·40m	−11·30m	−11·40m	−10·10m	−10·3m	−10·50m
245—247	−9·31sh	−9·95sh	−9·50sh	−7·24sh	−7·26sh	−7·52sh

EXPERIMENTAL

Materials.—Vitamin B_{12} was from Glaxo Research Ltd. The other corrinoids were prepared from it by literature methods.[2,6,8,9] Dilute solutions of hydrogen cyanide in chloroform and in iso-octane were prepared by shaking the organic solvent (*ca.* 50 ml) with a few drops of aqueous 4% hydrogen cyanide, and then drying the product.

[9] R. Bonnett, J. R. Cannon, V. M. Clark, A. W. Johnson, L. F. J. Parker, E. Lester Smith, and Sir Alexander Todd, *J. Chem. Soc.*, 1957, 1158.

Estimation of Corrinoids.—The estimation of corrinoid polyamides is complicated by the variable hydration of these substances. In general, concentrations were estimated spectroscopically by use of the following ε values, which refer to the dicyanides. Cobalamin, cobinamide, cobyric acid, and other compounds of the normal series: λ 367 nm, ε 30,400; [10] 13-*epi*-cobalamin and other compounds of the neo-series: λ 367 nm, ε 20,600; [2] dehydrovitamin B_{12}: λ 366 nm, ε 23,000; [9] vitamin B_{12} lactone: λ 368 nm, ε 26,300; [9] 10-chlorodehydrovitamin B_{12}: λ 369 nm, ε 28,200 [9] (this value was also used for the vitamin B_{12} chloro-lactone); corrinoid penta- and hexa-carboxylic acids: λ 365 nm, ε 27,000; [8] heptamethyl dicyanocobyrinate: λ 369 nm, ε 32,400; heptamethyl dicyano-13-*epi*-cobyrinate: λ 367 nm, ε 21,400. These values for the esters refer [2] to aqueous ethanolic (10 : 1 v/v) solutions 0·01M in potassium cyanide, but were also employed for the other solvents used.

C.d. Curves.—These were recorded on a Roussel–Jouan Dichrographe 185 at a concentration of *ca.* 0·1 mg ml^{-1}, with path length 1 cm or 1 mm. Low temperature c.d. measurements were made with the manufacturers' low temperature cell over the range −10 to −180° in either methanol–ethanol (1 : 4) or ether–isopentane–ethanol (5 : 5 : 2). Δε Values are corrected for the contraction in volume on cooling.[11]

We thank the Petroleum Research Fund, administered by the American Chemical Society, and the S.R.C. for support; Dr. D. G. Redman for preparing some of the samples; Dr. H. A. O. Hill and Dr. R. J. P. Williams for correspondence and discussions; and Professor W. Klyne for his continued interest.

[2/1748 *Received*, 25*th July*, 1972]

[10] H. A. O. Hill, J. M. Pratt, and R. J. P. Williams, *J. Chem. Soc.*, 1964, 5149.
[11] R. Passerini and I. G. Ross, *J. Sci. Instr.*, 1953, **30**, 274.