DNPHs of α-alkyl substituted saturated carbonyls showed higher R_F values than other DNPHs having the same chain lengths. The results obtained with the two types of plate did not show any marked differences. Separation was also possible on non-impregnated plates, but here the R_F value differences were smaller than with the impregnated plates.

It is immaterial whether the α-alkyl substituent is a methyl or an ethyl group. At the time, corresponding aldehyde-DNPHs with an even longer side chain at the α-position, and comparable ketone-DNPHs, were not available. A ketone-DNPH having two substituents at the α-position (3,3-dimethyl 2-butanone) had an R_F value which differed appreciably from that of the normal ketone-DNPH. DNPHs of saturated ketones with one substituent at the α-position can probably also be separated on silver nitrate plates from other ketone-DNPHs having the same chain length.

We have also compared 2-methyl cyclopentanone-DNPH with the DNPHs of 3-methyl cyclopentanone, cyclopentanone, and cyclohexanone. Surprisingly, the DNPHs of cyclic ketones were absorbed much more strongly on silver nitrate plates than DNPHs of aliphatic ketones. 2-Methyl cyclopentanone-DNPH, however, migrated much faster than the other cyclic ketone-DNPHs. This difference was less pronounced on plates not impregnated with silver nitrate.

We suppose that the bond between the C=N bond and the silver nitrate is weakened by the α-alkyl substituent, as a result of which separation becomes possible.

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Thin-layer chromatography of cyanocobalamin, hydroxocobalamin and B_{12} coenzymes

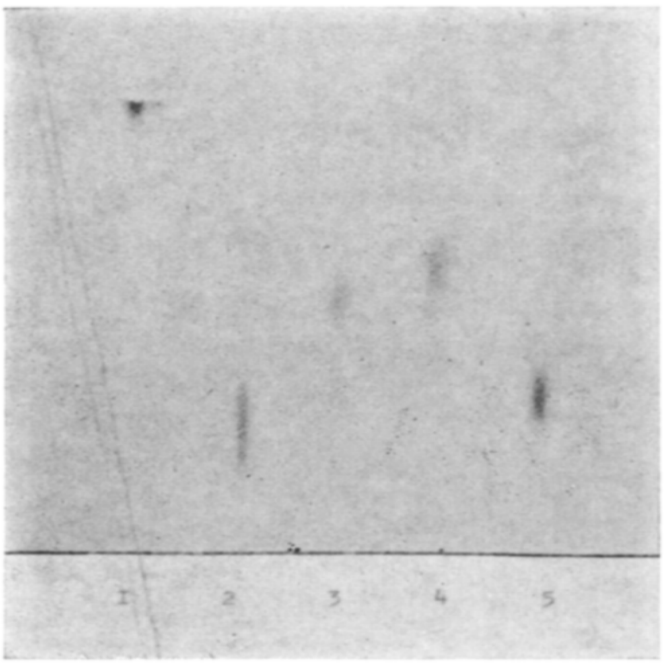
Vitamin B_{12} vitamers and their coenzyme forms can be separated by paper partition chromatography and paper electrophoresis. Paper partition chromatography takes a long time for the development and a shortcoming is that spots tail. On the other hand, paper electrophoresis is performed within a comparatively short time and has a good resolution. However, with the latter method not many samples can be examined at the same time a factor which is very desirable during the preparation and purification of such coenzyme forms of the vitamin.

Application of thin-layer chromatography for the separation of cyanocobalamin, hydroxocobalamin etc. was reported by CIMA AND MANTOVAN[1] and KAMIKUBO AND HAYASHI[2].

The present communication reports the separation by thin-layer chromatography of cyanocobalamin (CN-B_{12}), hydroxocobalamin (OH-B_{12}), adeninylcobamide coenzyme (ACC), benzimidazolylcobamide coenzyme (BCC) and dimethylbenzimidazolylcobamine coenzyme (DBCC), which can be done within a very short time with a good resolution.

CN-B_{12} and OH-B_{12} were obtained from Kaken Chemicals Co. and Roussel Co., respectively. DBCC was prepared by the following procedure: *Propionibacterium shermanii* IAM 1725 was grown in a medium containing the following ingredients per liter: glucose, 20 g; meat extract, 35 g; yeast extract, 5 g; $Na_2HPO_4 \cdot 12\,H_2O$, 1.75 g; K_2HPO_4, 1.75 g; $MgSO_4 \cdot 7H_2O$, 400 mg; $FeSO_4 \cdot 7H_2O$, 10 mg; $CoCl_2 \cdot 6H_2O$, 5 mg; calcium pantothenate, 4 mg; biotin, 0.3 mg. Incubation was carried out, stationary, at 30°. After the maximum growth was attained, 12.5 mg of CN-B_{12} and 5 g of glucose were added per liter of the medium, and the incubation was continued for a further 5 days. The cells were harvested by centrifugation and washed twice with 0.1 *M* phosphate buffer, pH 7.0. These cells were extracted with 80% ethanol at 82° for 30 min. The cells were filtered off and the orange-red colored filtrate was evaporated to dryness on a water bath at 50°. The dried residue was dissolved in water, followed by the addition of phenol. The orange-red color was transferred into the heavier phenol phase, which was displaced back into water when the phenol was removed by shaking with ether. Then, the colored solution was passed through a column of DEAE-Sephadex, A-25, equilibrated with 0.01 *M* sodium acetate. The effluents containing the color were combined and buffered with acetate buffer, pH 3.5, to give a concentration of 0.04 *M*, which was subsequently placed on a P-cellulose column equilibrated with 0.04 *M* acetate buffer, pH 3.5. The column was eluted first with the same buffer and next with 0.04 *M* acetate buffer, pH 4.7. The red fractions eluted were combined and extracted by using phenol and water as above. All operations were conducted with the maximal exclusion of light. Finally, red crystals were obtained by lyophilization which showed coenzyme B_{12} activity as measured by the formation of propionaldehyde from propanediol[3] with cell-free extracts of *Aerobacter aerogenes* ATCC 8308. The absorption spectra[4] over the range of 220 to 600 mμ in the absence and presence of 0.1 *M* KCN and electrophoretic behavior[5] agreed entirely with the described characteristics of DBCC. Additional confirmatory evidence of the identity of DBCC was obtained by the exposure of the compound to visible light which led to the degradation to OH-B_{12}. ACC was prepared from *Prop. arabinosum* IAM 1714 cells by a similar procedure to that described above except that no CN-B_{12} was added as the precursor. Identification of ACC was made by a similar method to that employed for DBCC. BCC was a gift from Prof. H. A. BARKER.

The CM-cellulose plates were prepared with MN300 and MN300CM (products of Macherey, Nagel & Co.). The developing solvent system was the lower layer of the mixture of *sec.*-butanol, 0.1 *M* acetate buffer, pH 3.5, and methanol (4:12:1) which gave a most satisfactory separation. Fig. 1 shows the result. The compounds could be sharply separated by this system, the R_F value of ACC differing particularly greatly from those of DBCC and BCC. Fig. 2 also shows the result of the exposure of DBCC to visible light by which it was degraded to OH-B_{12}.



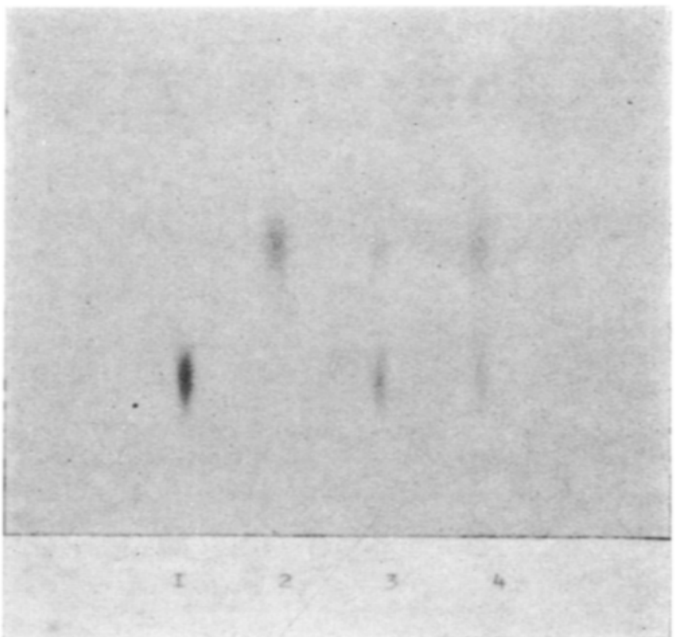


Fig. 1. Thin-layer chromatogram of B_{12} vitamers and coenzymes. Length of run was about 10 cm. 1 = CN-B_{12}; 2 = ACC; 3 = BCC; 4 = DBCC; 5 = OH-B_{12}.

Fig. 2. Thin-layer chromatogram of DBCC with and without exposure to visible light. Aliquots of a DBCC solution were exposed for 30 min to a 500-watt tungsten filament lamp at a distance of 30 cm. The exposure was made in a normal and an amber glass tube kept at 0°. Length of run was about 10 cm. 1 = OH-B_{12}; 2 = DBCC; 3 = DBCC exposed to light in a normal tube; 4 = DBCC exposed to light in an amber tube.

It should be added that in this chromatographic method it is essential that the inorganic ions are removed from the samples in order to obtain a good resolution, since CM-cellulose is a cation-exchanger. The removal can be easily accomplished by washing the phenol phase with water.

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