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Note

Thin-layer chromatography of acid-labile cobalamins

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Because of the complex structure of corrinoids, the common methods of analysis, *viz.*, nuclear magnetic resonance spectroscopy, infrared spectroscopy, and elemental analysis, are not very informative with these compounds and much of corrinoid chemistry relies on optical absorption and chromatographic data. The relatively few reports concerning the separation of corrinoids have dealt mostly with natural vitamin B_{12} derivatives[1–7]. Firth *et al.*[8] have used cellulose thin-layer chromatography (TLC) to separate a variety of synthetic cobalamines.

Because of our interest in the mechanism of action of coenzyme B_{12}-dependent enzymes, we have synthesized and studied reactions of analogues of alkylcobalamins[9,10]. Many of these compounds are quite similar in structure and some are very acid labile. We wish to report the separation of these cobalamins by TLC on cellulose under slightly basic conditions.

EXPERIMENTAL

5'-Deoxyadenosylcobalamin was a gift of Professor Robert Abeles, Department of Biochemistry, Brandeis University, U.S.A., and cyanocobalamin a gift of Merck Co., Rahway, NJ, U.S.A. 2,3-Dihydroxypropyl-, 1,3-dioxa-2-cyclopentylmethyl-, 2,2-diethoxyethyl-, and formylmethylcobalamin were prepared as previously reported[9,10]. Methylcobalamin was prepared by the method of Dolphin[11]. Hydroxocobalamin was synthesized as previously reported[9]. The compounds were dissolved in 0.5% concentrated aqueous ammonia and applied to 20 cm long, 0.1 mm thick microcrystalline cellulose plates (E. Merck, distributed by Brinkmann, Westbury, NY, U.S.A., as Celplate-22 without indicator). Ascending TLC was performed at ambient temperature in the dark for a distance of at least 10 cm, eluting with *n*-butanol–ethanol–water, (10:3:7) containing 0.5% concentrated aqueous ammonia. Because of the intense red color of these compounds, visual detection was employed.

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RESULTS

Table I summarizes the R_F values for the various axial ligand-substituted cobalamins. The two R_F values given for 2, 3 dihydroxypropylcobalamin are for the two compounds which result from the synthesis previously described[10]. These two compounds may be the two diastereomeric forms of 2,3-dihydroxypropylcobalamin.

As Firth *et al.*[8] found previously, small changes in the structure of the axial ligand dramatically effect the mobility of cobalamins on cellulose.

TABLE I

R_F VALUES FOR AXIAL LIGAND SUBSTITUTED COBALAMINS

Axial ligand-cobalamin	R_F
2,2-Diethoxyethyl-	0.62
Methyl-	0.60
1,3-Dioxa-2-cyclopentylmethyl-	0.56
Formylmethyl-	0.51
Cyano-	0.48
2,3-Dihydroxypropyl-	0.46
	0.38
5'-Deoxyadenosyl-	0.34
Hydroxo-	0.22

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