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EFFECT OF AGLYCONE ON THE RATE OF CYANIDE CLEAVAGE OF Co-C-BOND IN NUCLEOSIDE ANALOGUES OF COBAMIDE COENZYME.

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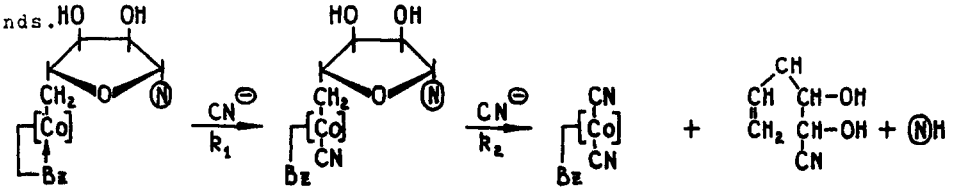
The cyanation reaction may be a convenient model for a quantitative estimation of the trans-effects in cobalamins .In the present work such approach has been used for investigating the aglycone effect in the deoxynucleosidic part on the stability of the Co-C and Co-Bz bonds in α-(5,6-dimethylbenzimidazolyl)cobamide coenzyme (DBCC) and its analogues obtained from the vitamin B_{12s} and 2',3'-phenylboronic esters of 5'-O-tosylnucleosides [1,2].It is known that the reaction of cyanide with 5'-deoxynucleosylcobalamins in dark cleaves simultaneously the Co-C and glycoside bonds affording purine and pyrimidine [3].Spectrophotometric investigation of cyanide cleavage of DBCC and its analogues carried out by the authors [4] showed that the reaction is a two-step process.The fast step (k_1) involves a substitution of the "lower" benzimidazol ligand while the slow one (k_2) is cleavage of the Co-C and glycoside bonds.

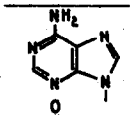
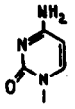
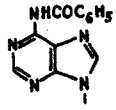
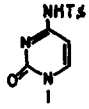


The abbreviations used are : vitamin B_{12}, cyanocobalamin ;vitamin B_{12s},reduced form of the vitamin B_{12} containing monovalent cobalt, (Co^{I}).

The absorbtion spectra of DBCC and its analogues were taken at different moments of the second stage.They revealed the isobestic points (348 and 535nm) convenient for the measurement.The kinetics were studied at a large molar excess of $CN^{\ominus}$, thus both the steps are pseudomonomolecular.The values of k_1 and k_2 for DBCC and its analogues along with other properties of the compounds are shown in the Table.

Table

Properties of 5'-deoxynucleosylcobalamines.

Aglycone	Relative electrophoresis mobility[a]	UV-spectrum λ_{max} (nm)	UV-spectrum $\varepsilon \times 10^{-4}$ $M^{-1}cm^{-1}$	Molecular ellipticity λ_{max}	Molecular ellipticity $[\theta]_\lambda \times 10^{-4}$ deg.cm^2 mole^{-1}	Cyanation rate constants[b] $k_1 \times 10^2$ sec^{-1}	Cyanation rate constants[b] $k_2 \times 10^4$ sec^{-1}
	1.0	262	3.83	298 268	+2.0 +3.5	2.9±0.1[+]	8.4±0.4
	0.67	256	3.00	300 270 250	+1.6 +1.6 +2.1	2.4±0.1	2.9±0.1
	1.14	268	2.81	300 286 270 258 240	+1.5 -0.4 +1.3 +0.9 +2.2	1.7±0.2	3.5±0.2
	0.57	282	3.72	297 276 254	+2.0 +1.8 -2.3	2.6±0.1	0.44±0.02
	0.56	268 282	2.87 2.92	300 266 230	+0.9 +3.2 -3.0	-	0.97±0.05

a) 0.5 N acetic acid. b) Reaction with 0.1 M KCN, 25°C, sodium bicarbonate buffer, pH 10.5.

We also studied the effect of an aglycone substitution on the strength of "lower" axial ligand - cobalt coordination bond (trans-effect).

[+] The Schrauzer k value for a DBCC cyanation [5] corresponds to the k_1 of the present work.

Fig.1 shows the pK_a of ionization of 5'-deoxyadenosylcobalamin vs the rate constant of the benzimidazol substitution by cyanide (k_1).The pK_a's of DBCC and its analogues have been found earlier by spectrophotometric titration [2]. Aglycone substitution in the nucleoside ligand affects the k_2 of cyanation as well.A change of the Co-C bond stability in the cobalamin might be due to the changes in its polarization or conformation which could be found by the spectral investigation.The UV and visible spectra of cobamide coenzyme analogues are similar to that of DBCC [6].This similarity is observed only in the region of 320-600 nm.A hypsochromic shift of absorbtion is always observed (assigned to the 7π -8π-transition [7]) from 525-530 nm(pH7.8) to 460 nm(pH1.3). In the region of 230-300 nm the absorbtion bands shift upon the aglycone substitution in the "upper" nucleoside ligand.The pattern of CD curves for DBCC and its analogues also confirms this similarity.At 300-600 nm the CD curves of coenzyme and its analogues are practically identical while at 230-300 nm they differ to some extent (see the Table).

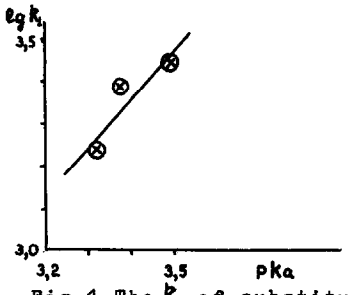


Fig.1.The k_1 of substitution of the "lower" ligand vs pK_a of ionization of 5'-deoxynucleosylcobalamins .

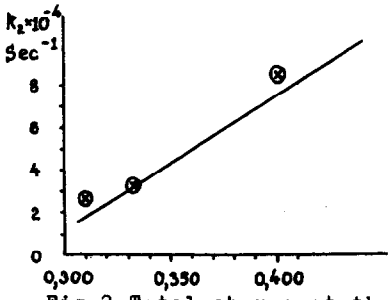


Fig.2.Total charge at the glycoside nitrogen of ribonucleotides vs k_2 of cyanation of 5'-deoxynucleosylcobalamins .

The greatest difference is observed in the CD curve of a cytidyl analogue. Although the 260-270 nm absorbtion of vitamin B_{12} is assigned to 7π -10π-transition [7] it should be noted that it is hardly believable that such interpretation is unequivocal in case of DBCC and its analogues.Meanwhile a similarity of the CD curves for coenzyme and its analogues within the region of 300-600 nm indicates at the absence of conformation changes upon adenine substitution in DBCC by other aglycone.These results show that an electron-att-

ractive substituent in the purine nucleus stabilizes the Co-C bond.It was found that the 6-N-benzoyl coenzyme derivative undergoes slow cleavage.An analogous effect was observed on the introduction of a p-toluenesulfonyl group into the cytosine amino group of the cytidyl analogue of the coenzyme.A good correlation between the total positive charges (Pullman [8]) at the glycoside nitrogens of ribonucleotides and the cleavage constants of an organometallic bond of the analogues (Fig.2) allows us to assume a type of hyperconjugation between the heterocycle and the metal atom.It is known that the Co-C bond does not cleave under cyanation of the alkyl cobamide coenzyme derivatives. The apurine analogue of DBCC - 6-deoxyglucosylcobalamin obtained by treatment of 6-O-tosylglucopyranose [9] or 6-bromo-6-deoxyglucose [10] with vitamin B_{12s} is not subject to cleavage by cyanide in the dark.The consideration of the results obtained shows that the bond between central cobalt atom and the "upper" and "lower" axial ligands sterically remote and formally non-conjugated with adenine fragment is rather sensible toward chemical changes in the purine nucleus.This is in turn evidence of a specific role of the adenine fragment in cobamide coenzyme which determines some of its chemical and probably biocatalytic properties.

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