α-(5,6-Dimethylbenzimidazolyl)rhodibamide and Rhodibinamide, the Rhodium Anallogues of Vitamin B\textsubscript{12} and Cobinamide*

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VOLKER B. KOPPENHAGEN, FRITZ WAGNER, AND JOSEPH J. PFIFFNER

From the Institut für Molekularbiologische Forschung, 3301 Stiickheim, West Germany, and the Department of Physiology and Pharmacology, Wayne State University School of Medicine, Detroit, Michigan 48201

SUMMARY

Monocyano-α-(5,6-dimethylbenzimidazolyl)rhodibamide, dicyano-α-(5,6-dimethylbenzimidazolyl)rhodibamide, and dicyanorhodibinamide have been prepared by insertion of rhodium into the metal-free analogue of vitamin B\textsubscript{12} using rhodium carbonyl chloride. The monocyano form of the rhodium analogue of vitamin B\textsubscript{12} was also obtained by treatment of the corresponding dicyano form with silver nitrate. The new compounds are characterized by their spectral and electrophoretic properties and their biological activity. While dicyano-α-(5,6-dimethylbenzimidazolyl)rhodibamide is biologically inactive, the corresponding monocyano form is active as antimetabolite to vitamin B\textsubscript{12} in suppressing the growth of Lactobacillus leichmanii (ATCC 7830).

The only metals so far incorporated into naturally occurring descobaltocorrinoids are cobalt (3-5), copper (5, 6), and zinc (5, 6). Attempts to insert other metals have been without success, although the insertion of metals into a synthetic corrin has not presented any major difficulty (7). Thus Eschenmoser has reported the successful incorporation of cobalt, nickel, palladium, rhodium, zinc, and lithium into the metal-free 1,2,2,7,7,12,12-heptamethyl-15-cyan-corrin hybrobromide (7). Of principal interest in the natural descobaltocorrinoid series is the insertion of a transition metal, which would allow the synthesis of the corresponding cobamide coenzyme analogue. Such an analogue may be an interesting inhibitor of vitamin B\textsubscript{12} coenzyme. We wish to report the preparation of α-(5,6-dimethylbenzimidazolyl)rhodibamide and rhodibinamide, the rhodium analogues of vitamin B\textsubscript{12} and cobinamide.

The metal-free analogue of vitamin B\textsubscript{12} was prepared from Chromatium (ATCC 17899), which was grown as described previously (6). The method first used to isolate and purify this compound involved repeated extraction with phenol, ion exchange treatment, and paper electrophoresis at pH 2.5 (6). Presently the aqueous extract of Chromatium is passed through a column of XAD-2 and the retained descobaltocorrinoids are eluted separately with aqueous tert-butyl alcohol (8), as outlined in Fig. 1. The descobaltocobalamin thus obtained still contains small amounts of phenylhydrogenobamide. The compound is therefore retained on a small bed of CM-cellulose in the hydrogen form, eluted with 0.5 N acetic acid, and recycled through a small column of XAD-2. The yield of α-(5,6-dimethylbenzimidazolyl)hydrogenobamide is 0.7 μmole/100 g of wet cells.

Rhodium Corrinoids—The rhodium analogues of vitamin B\textsubscript{12} and cobinamide were prepared by insertion of rhodium into the metal-free analogue of vitamin B\textsubscript{12} using rhodium carbonyl chloride [Rh(CO)\textsubscript{5}Cl\textsubscript{2}]. The use of this reagent has been indicated for the insertion of rhodium into a synthetic corrin (7) and for the preparation of rhodium porphyrins (9). A solution of α-(5,6-dimethylbenzimidazolyl)hydrogenobamide (30 mg) in ethanol-glacial acetic acid (3:1, v/v) (30 ml) was allowed to react with rhodium carbonyl chloride (150 mg) for 24 hours at 1 The abbreviations used are: XAD-2, Amberlite XAD-2; rhodibalamin, α-(5,6-dimethylbenzimidazolyl)rhodibamide. The symbol [Rh] represents rhodobinamide.
room temperature. The reaction mixture was diluted with 150 ml of water and the pH was adjusted to 9.5 using solid KCN. Ethanol was distilled off under reduced pressure and the remaining solution was kept at room temperature for 10 hours. From the reaction product thus obtained rhodium corrinoids were isolated as outlined in Fig. 2. After the product was desalted by adsorption and elution using a column (2.2 x 6 cm) of XAD-2 (50 to 100 μm), basic corrinoids, mainly yellow descobalcoorinoids, were retained on a column (2.2 x 5 cm) of CM-cellulose in the hydrogen form. The aqueous pass-through contains a mixture of rhodium-containing corrinoids which was separated into a neutral and an acid fraction by treatment with DEAE-cellulose in the acetate form. Both fractions were chromatographed on columns (1.3 x 5 cm) of XAD-2 (30 to 50 μm) using aqueous tert-butyl alcohol as eluant. After elution of small amounts of rhodium-containing byproducts with 6 volume % tert-butyl alcohol, the neutral fraction was separated into two main compounds by elution with 8 and 10 volume % tert-butyl alcohol, while the acid fraction was eluted as a single compound with 10 volume % tert-butyl alcohol. The products thus obtained are rhodium corrinoids with properties expected for monorange-α-(5,6-dimethylbenzimidazolyl)rhodobalamin (neutral 8% zone), dicyanorhodobalamin (neutral 10% zone), and dicyano-α-(5,6-dimethylbenzimidazolyl)rhodobalamin (acid 10% zone). They appear homogeneous in paper chromatography and paper electrophoresis after they have been chromatographed on Whatman No. 3MM with water-saturated 2-butanol containing 0.02% HCN as the solvent system. Cyanorhodobalamin and dicyanorhodobinamide were crystallized by addition of 10 volumes of acetone to a concentrated aqueous solution. The former forms thin orange-red and the latter deep red needles. The total yield of rhodium-containing corrinoids is about 42%. The relative yields are as follows: cyanorhodobalamin (9%), dicyanorhodobinamide (30%), and dicyanorhodobinamide (71%).

The structural relation among the three compounds was established by the following reactions: cerous hydroxide hydrolysis of dicyanorhodobalamin according to the method of Friedrich and Bernhauer (10) yields equimolar amounts of dicyanorhodobinamide and α-ribazole. The latter was identified by ultraviolet spectroscopy, paper chromatography, and paper electrophoresis. The dicyanorhodobinamide obtained is identical with the neutral 10% zone (Fig. 2).

Treatment of dicyanorhodobalamin with silver nitrate gives in almost quantitative yield the monocyanoform of rhodobalamin. An aqueous solution of the acid 10% zone was allowed to react with silver nitrate for 10 hours at room temperature. The identity of the formed product with the neutral 8% zone (Fig. 2) was established, after it was desalted by XAD-2 treatment and passed through a small bed of DEAE-cellulose in the acetate form (see Scheme 1).

All three compounds were found to contain 1 mole of rhodium, as determined by atomic absorption. Based on the similarity of their absorption spectra (Figs. 4 and 5) with those of the corresponding CoIII analogues the RhIII oxidation state is assigned to the rhodium in these complexes. The presence of the cyan ligands is indicated by the infrared spectra (Fig. 3) of cyanorhodobalamin, dicyanorhodobalamin, dicyanorhodobinamide, and aquocyanorhodobinamide, which show absorption maxima at 2137 cm⁻¹, 2119 cm⁻¹, 2119 cm⁻¹, and 2133 cm⁻¹, respectively. The differences in the stretching frequencies of the cyano ligand can be attributed to the different axial ligand in the second axial position. The axial ligand becomes a better donor (6,6-dimethylbenzimidazole < OH⁻ < CN⁻), the stretching frequency approaches the value for cyanide ion (2079 cm⁻¹). The same effect has been observed in the corresponding cobalt corrinoid series (11). The similarity of the spectrum of cyanorhodobalamin with that of cyanocobalamin further indicates an identical structure of the peripheral corrin moiety of both compounds.

The assigned structures are further confirmed by the electrophoretic behavior and the absorption spectra of these compounds. Monocyancorhodobinamide and dicyanorhodinamide are neutral in 0.1 N KCN, at pH 7 and 2.7. Dicyanorhodobinamin is negatively charged in 0.1 N KCN and at pH 7, and it is neutral at pH 2.7. The charge properties are explained by the formulae in Scheme 2. The neutral behaviour of dicyanorhodobinamide at pH 2.7 indicates that the second cyano group is not exchanged as in the corresponding dicyanocobinamide. The neutral charge of monocyanorhodobinamin in 0.1 N KCN further indicates that the benzimidazole base is more strongly attached.
FIG. 3. Infrared absorption spectra of vitamin B$_{12}$ (- - -), mono cyanorhodibalamin (----), di cyanorhodinamide (-----), and cyanooaquorhodinamide (-----).

FIG. 4. Absorption spectra of dicyanorhodibalamin (2.54 × 10$^{-4}$ M) in water at pH 7 and 11 (-----) and at pH 1 (-----); monocyano rhodibalamin (2.53 × 10$^{-4}$ M) in water at pH 1 and 7 and in 0.1 N KCN (-----); di cyanocobalamin (2.91 × 10$^{-4}$ M) in 0.1 N KCN (-----).

to rhodium than to cobalt. Whereas cyanocobalamin becomes negatively charged in 0.1 N KCN due to the exchange of cyanide for benzimidazole, the rhodium complex remains neutral. With di cyanorhodibalamin the exchange of the cyano ligand by benzimidazole does not occur. The absorption spectra of monocyano and dicyanorhodibalamin and di cyanorhodinamide are shown in Figs. 4 and 5. They have the same general configuration of absorption bands which is characteristic of the corrinoids, two relatively weak bands close together in the visible region (α- and β-band) and an intense band in the upper ultraviolet region (γ-band). Based on the theoretical molecular weight of 1425 for dicyanorhodibalamin and 1399 for the corresponding monocyano form the millimolar extinction coefficients for the γ-bands are 33.8 (dicyano form at 350 nm) and 30.4 (monocyano form at 345 nm). The spectra of dicyanorhodibalamin and di cyanorhodinamide are identical in the region from 300 to 600 nm; the only difference is the appearance of a narrow band at 289 nm (pH 7 and 11) or 285 nm (pH 1) which can be attributed to the 5,6-dimethylbenzimidazole moiety. This difference is not as obvious because the spectrum of dicyanorhodinamide contains a band at 291 nm (pH 1, 7, and 11). In the curve of monocyano rhodibalamin the benzimidazole band is replaced by a shoulder at 286 nm (pH 1, 7, and 11) which is similar to the spectrum of the monocyano form of vitamin B$_{12}$, where the absence of this
band is explained by a coordinate linkage between N-3 of 5,6-dimethylbenzimidazole and cobalt (12). That the marked decrease in the resolution of the corresponding maximum in the spectrum of the rhodium analogue may be interpreted as a similar coordination is indicated by a hypsochromic shift of the main peaks in the visible and ultraviolet regions as compared with the corresponding bands in the spectrum of dicyanorhodobilamin at pH 1, 7, and 11 (528 nm → 514 nm, 497 nm → 486 nm, and 350 nm → 345 nm). Compared with the spectrum of dicyanorhodobilamin, the corresponding bands in the spectrum of dicyanorhodobilamin show a general hypsochromic shift of the $\alpha$-, $\beta$-, and $\gamma$-bands. Similar observations have been made in the coordination chemistry of metalloporphyrins where in the series of Co$^{III}$, Rh$^{III}$, and Ir$^{III}$ the band shift to shorter wave length increases in the order Ir > Rh > Co (9).

A striking property of the dicyanorhodium corrinoids is their conversion into yellow products at a pH below 2.5. If dicyanorhodobinamide is kept in 0.01 N HCl for 15 hours at room temperature a product is obtained with properties expected for cyanoaquorhodobinamide. The compound is neutral at pH 11 and positively charged at pH 6.5 and 2.5. The absorption spectrum (Fig. 5) shows a hypsochromic shift of the $\alpha$-, $\beta$-, and $\gamma$-bands as compared with the spectrum of dicyanorhodobilamin (528 nm → 499 nm, 497 nm → 481 nm, and 350 nm → 340 nm). At pH 11 these bands shift to longer wave length (499 nm → 510 nm, 497 nm → 492 nm, and 340 nm → 343 nm), which can be explained by the loss of 1 proton of the aquo ligand. This interpretation is confirmed by the neutral charge of this compound at pH 11. Addition of KCN reconverts this product into dicyanorhodobilamin.

The biological activity of mono- and dicyanorhodobilamin was tested with *Lactobacillus leichmanii* (ATCC 7830) according to the United States Pharmacopeia method (13). In the absence of vitamin B$_{12}$ neither compound showed significant growth-promoting activity. The activity was less than 0.005% that of cyanocobalamin. The highest level tested was 20 pmoles of rhodium corrinoid per ml of medium.

Further tests were carried out to determine whether these compounds exhibited any anti-vitamin B$_{12}$ activity. The effect was determined on a culture of *Lactobacillus leichmanii* (ATCC 7830) supplemented with cyanocobalamin and grown by the standard method (13). The vitamin B$_{12}$ concentration was 0.1 pmole per ml of test medium. While dicyanorhodobilamin showed no anti-vitamin effect, the corresponding monocyan form was active as an antimetabolite of vitamin B$_{12}$ with a 50% inhibition index of 65:1. The 50% inhibition index is defined as the ratio of inhibitor to cyanocobalamin which reduces the growth response to 50% of that obtained with the vitamin alone.

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Volker B. Koppenhagen, Fritz Wagner and Joseph J. Pfiffner

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