183. Structure and Reactivity of Xanthocorrinoids.

Part II[1])

Influence of the *c*-Acetic-Acid Chain on the Course of the Hydroxylation of the Corrin Chromophore by Oxygen in the Presence of Ascorbic Acid

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Dedicated to Prof. *Albert Eschenmoser* on the occasion of his 60th birthday

(28.5.85)

The yield of the main product from the reaction of heptamethyl dicyanocobyrinate with O_2 in the presence of ascorbic acid has been optimised by systematic variation of the reaction conditions, and the structure of the obtained 'stable yellow corrinoid' has been established by X-ray analysis. From the relationship between the structures of analogous xanthocorrinoids obtained likewise from a series of dicyanocobyrinic-acid derivatives, which has been prepared by systematic modifications of the *c*-acetic-acid chain, and the functionality of the substituent at C(7), a reaction mechanism is suggested.

Stable yellow corrinoids, also termed 'xanthocorrinoids' [2], are formed as by-products of most reactions of cyanocobalamin (vitamin B_{12}) and its derivatives, which proceed with change of the oxidation state of the complex-bonded Co ion [3b]. They are stable to air and to light, and show, as a common feature, absorption bands at about 460–480 nm, a spectral range which is also characteristic for some yellow compounds formed on degradation of vitamin B_{12} by bacteria (*e.g. Aerobacter aerogenes* [4] and *Pseudomonas rubescens* [5–9]). Moreover, metal-free yellow corrinoids have been obtained from the culture filtrates of phototrophic bacteria as *Rhodopseudomonas sphaeroides* or *Rh. capsulata,* when grown in a medium deficient in Co [10]. However, as the structures of the above mentioned xanthocorrinoids are, with one exception, unknown, it is questionable whether all of them belong to the same class of compounds.

Among the different reactions, which lead to the formation of stable yellow corrinoids from vitamin-B_{12} derivatives *(cf. Fig. 1),* we investigated first the formation of such pigments under the conditions of *Udenfriend*'s reaction using heptamethyl *Coα, Coβ*-dicyanocobyrinate (**1**) as a substrate. Now, after a series of experiments directed towards the elucidation of the mechanism of the formation of xanthocorrinoids using different dicyanocobyrinic-acid derivatives as substrates has been completed [11], the experimental

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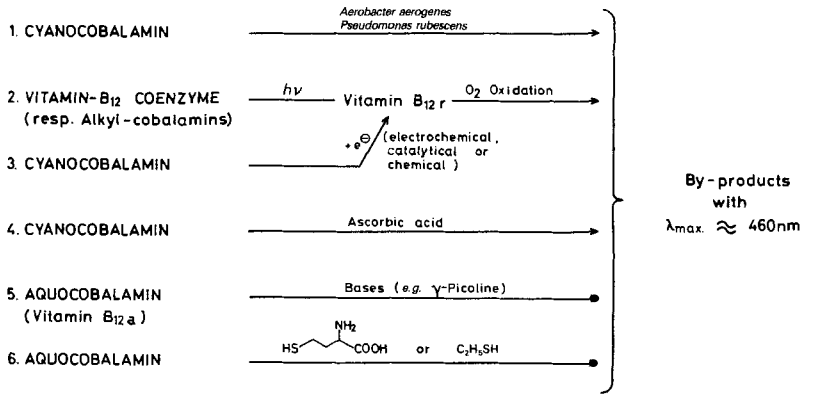


Fig. 1. *Formation of stable yellow corrinoids.* For references, see [3].

Scheme 1

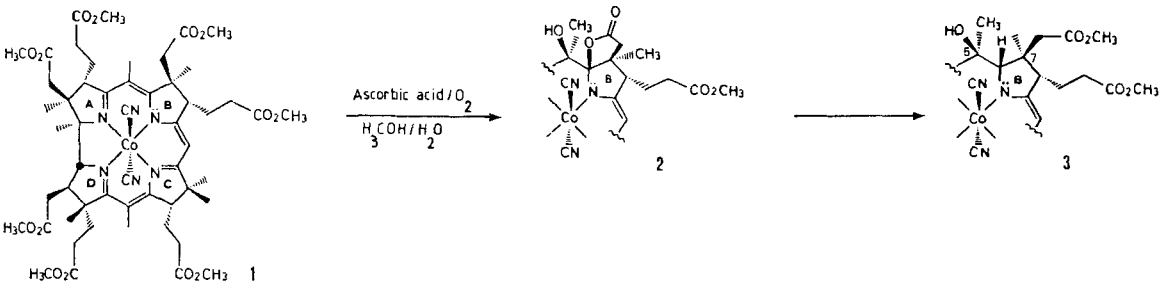


Table 1. *Dependence of Yield of the Yellow Corrinoid on Reaction Time, H^+-Concentration, Reaction Temperature, and Molar Ratio* n *of Reactants*[a])

t [h]	Yellow Corrinoid [%]	Recovered **1** [%]	Conversion [%]	pH	Yellow Corrinoid [%]	Recovered **1** [%]	Conversion [%]
1	8	81	42	5.5	0	40	0
2	15	65	43	6.5	22	41	37
2½	23	56	52	7.0	30	48	58
3	30	48	58	7.5	25	50	50
3½	30	40	50	8.0	13	35	20
4	23	20	29				
6	12	4	13				

T [°C]	Yellow Corrinoid [%]	Recovered **1** [%]	Conversion [%]	*n*[b])	Yellow Corrinoid [%]	Recovered **1** [%]	Conversion [%]
50	5	80	25	1:1	0	95	0
65	30	48	58	1:5	0	95	0
80	10	0	10	1:40[c])	30	48	58
100	0	0	0	1:90	30	48	58
				1:180	30	48	58

[a]) In MeOH/H_2O 1:2, bubbling O_2 in the presence of EDTA (0.01M).
[b]) *n* = Molar ratio **1**/ascorbic acid.
[c]) Between *n* = 1:5 and 1:40, the yields were not reproducible.

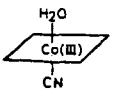
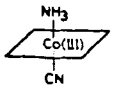
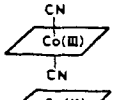
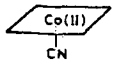
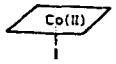
details of our work, shortly communicated some years ago [1] [12], are reported in the present paper.

Fenton's and *Udenfriend*'s reagents[5]) belong to the various model oxidation systems for enzymatic hydroxylations which have been explored during the past three decades [14]. We felt, therefore, that the products formed from dicyanocobyrinic acid could be of interest in connection with the bacterial degration of corrinoids. Actually, H_2O_2 which is probably formed by reduction of O_2 by ascorbic acid under the conditions of the *Udenfriend* reaction [15] is supposed to be involved in the aerobic degradation of aquocobalamin by *Streptomyces mitis* [16]. On the other hand, the lability of vitamin B_{12} and its derivatives against ascorbic acid (vitamin C) *in vitro*, first observed by *Gakenheimer* and *Feller* in 1949 [17] attracts considerable interest both from the analytical and the pharmacological point of view [3b] [18].

To improve the yield of the primary main product of the reaction of **1** with ascorbic acid, the influence of the reaction conditions on the yield was investigated systematically. From these experiments, which are summarized in *Table 1*, it follows that the optimum conditions for the formation of the main product are a large excess of ascorbic acid (at least 40 mol) in MeOH/H_2O at 65°C and pH 7.0 for 3 h. In agreement with earlier observations [19], the corrin ring is destroyed by ascorbic acid in acidic medium. Both an increase of the temperature above 65°C and of the reaction time beyond 3 h result in a diminution of the yield of yellow corrinoid, so the latter must be susceptible to further transformations by the reagents. As a matter of fact, the isolated compound is transformed under the same conditions which lead to its formation into a complex mixture of products, which have not yet been investigated in detail.

To obtain some information concerning a plausible mechanism of the yellow corrinoid formation, the dependence of the yield on the axial ligands of cobyrinic acid

Table 2. *Dependence of Yield of Yellow Corrinoid on Ligand Substitution and Oxidation State of the Co-Atom*

Starting material	Time[a])	Yellow corrinoid [%][b])	Recov. starting material [%]	Conversion [%]
	45 min	15	40	25
	2 h	25	29	35
	3 h	30	48	58
	30 min	9	36	14
	30 min	15	32	22

[a]) In MeOH/H_2O 1:2, bubbling O_2 in the presence of EDTA (0.01M).

[b]) At the end of the reaction time, the mixture was treated with CN^- ions to obtain the corresponding dicyanoxanthocorrinoid.

[5]) *Fenton*'s reagent consists of a mixture of Fe^{2+} ion and H_2O_2. The latter is replaced by a mixture of ascorbic acid, EDTA and O_2 in *Udenfriend*'s reagent [13].

heptamethyl ester was investigated next (see *Table 2*). As the reaction time quoted in *Table 2* for every metal complex corresponds to the highest yield of yellow corrinoid obtained under the optimum conditions established before, the conversion ratios reflect the reaction rates for the consumption of substrate. As in the case of vitamin B_{12} [19], the dicyano complex displays the highest stability against *Udenfriend's* reagent and affords the best yield of yellow corrinoid. It is noteworthy that the two Co(II) complexes which were investigated afforded only poor yields of yellow corrinoid. Thus, the reduction of the Co(III) ion of **1** is not likely to be the rate-determining step of its transformation into the yellow corrinoid, although the formation of Co(II) species in the course of the reaction, as it has been suggested in the case of vitamin B_{12} [20], cannot be ruled out on the basis of these experiments. In fact, when heptamethyl *Coα*, *Coβ*-diiodocob(III)yrinate was used as the substrate, the colour of the solution changed instantaneously from brownish red to dark green, thus indicating that reduction to the iodocobalt(II) complex had occurred.

As mentioned before, Fe^{2+} ions are essential in both *Fenton's* and *Udenfriend's* systems. In the case of heptamethyl *Coα*, *Coβ*-dicyanocobyrinate (**1**), however, we observed that in presence of Fe^{2+} or Cu^{2+} ions the decomposition of the yellow corrinoid formed initially was accelerated whereas its formation rate remained almost unchanged. Therefore, the best yields were obtained in the absence of metal ions (see *Table 3*). This result suggests that for the formation of the primary product, the complexed Co-ion in the substrate may assume the role of the metal ion in the conventional *Udenfriend* system (see *Scheme 4*). In the presence of EDTA, which is supposed to reduce the oxidation potential of the Fe^{2+}/Fe^{3+} couple in the *Udenfriend* system [21], the otherwise erratic yield of yellow corrinoid from **1** became reproducible. The same result could be obtained using an ion-free medium *(Exper.6* in *Table 3)*. On the other hand, substitution of a dilute solution of H_2O_2 (*Fenton's* system) for ascorbic acid and O_2 afforded the same yellow corrinoid, essentially in about the same yield (see *Exper. Part*).

Eight years ago, conventional spectroscopic methods proved to be insufficient for the elucidation of the structure of the yellow corrinoid obtained from **1** as described above. The detection of the parent peak by mass spectrometry which failed when using conventional ionization methods, was only achieved more recently by means of the FAB technique [22]. Clearly, the UV/VIS spectrum suggested that the conjugated system of double bonds in the corrin chromophore had been interrupted [4]. The α-, β-, and γ-bands, which are typical for the corrin chromophore, are still perceptible. However, in the visible range, the absorption bands are shifted hypsochromically by *ca.* 100 nm. The γ-band is shifted by 47 nm and its intensity is considerably decreased *(cf. Exper. Part)*. In

Table 3. *Dependence of Yield of Yellow Corrinoid on the Presence of Metal Ions*

Exp. No	Conditions	Time [h]	Yellow corrinoid [%]	Recovered **1** [%]	Conversion [%]
1	Fe^{2+}/EDTA (0.01M)	2	14	60	35
2	Fe^{2+}/EDTA (0.01M)	3	9	25	12
3	Cu^{2+}/EDTA (0.01M)	2	21	23	27
4	Cu^{2+}/EDTA (0.01M)	3	17	0	17
5	Distd. H_2O/EDTA (0.01M)	3	30	48	58
6	Redistd. H_2O	3	29	43	51

1972, *Pratt* had anticipated that the corrin ring might be hydroxylated by an oxidizing agent such as H_2O_2, HO_2^- or OH radicals [3b]. In agreement with this suggestion, the ^{1}H-NMR spectrum of the yellow corrinoid from **1** shows a *s* at 5.39 ppm corresponding to *one* proton, which is exchanged on addition of D_2O. However, the presence of an OH group could not be demonstrated by chemical methods. It is noteworthy, that the IR spectrum reveals the presence of an absorption band at 1785 cm^{-1}, characteristic for a five-membered cyclic lactone. Fortunately, attempts to crystallize the yellow compound were successful, so that its structure **2**[6]) could be established by X-ray analysis [1][7]).

From the chemical point of view, the most straightforward proof of structure **2** is the facile backtransformation into **1**. It is known [23] that the five-membered lactone ring of cyanocobalamin *c*-lactone can easily be opened by reduction with Zn in AcOH. Under the same conditions, the xanthocorrinoid **2** afforded, after treatment with CN^- ions and esterification with diazomethane, a mixture of **1** which proved to be identical with authentic material *(cf. Fig. 2)* and of a monohydro derivative **3**[6]) in 57 and 27% yield, respectively. The structure of the latter compound was confirmed *inter alia* by its spectro-

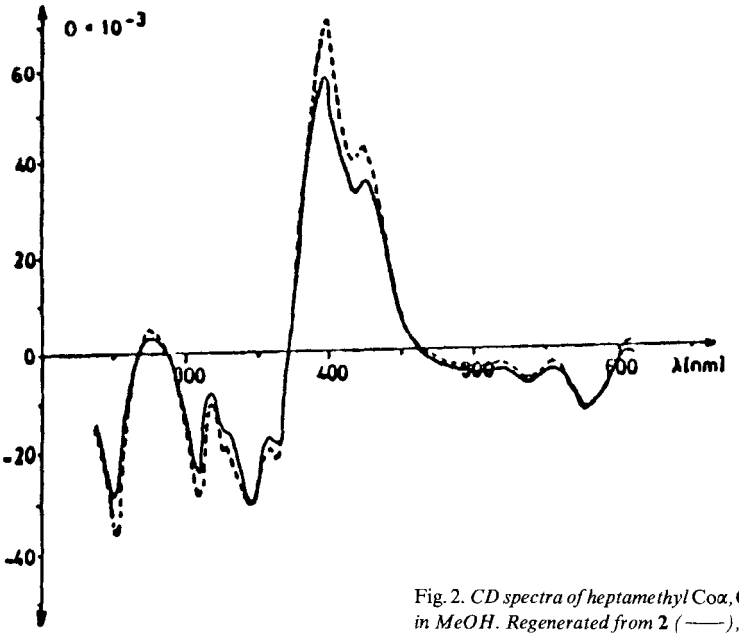


Fig. 2. *CD spectra of heptamethyl* Coα,Coβ*-dicyanocobyrinate* (**1**) *in MeOH. Regenerated from* **2** *(——), authentic material (---)*

[6]) For the sake of clarity, only the part of the molecule which is modified during the reaction is represented in the partial structure, remainder as in **1**.

[7]) From MeOH/Et_2O, xanthocorrinoid **2** crystallises together with one molecule of H_2O and one of MeOH in the orthorhombic space group $P2_12_12_1$ with $a = 1905.4$ (2), $b = 3141.6$ (2), $c = 954.0$ (1) pm; $Z = 4$, $d_{calc.} = 1.33$ g·cm^{-3}. The structure was solved by *Patterson* and difference syntheses and refined with the program SHELX-76 to the final reliability indices $R = 0.076$, $R_w = 0.081$ for 7657 independent reflections ($F_o^2 \geq 2.0\sigma(F_o^2)$, Cu$K\alpha$, $2\theta \leq 135°$). With the exception of the terminal methyl C-atoms of the side chains, all atoms were refined anisotropically. H-Atoms were not included in the refinement. (Lists of atom-positional parameters, bond lengths, and bond angles may be requested from Prof. *W. S. Sheldrick*, University of Kaiserslautern.)

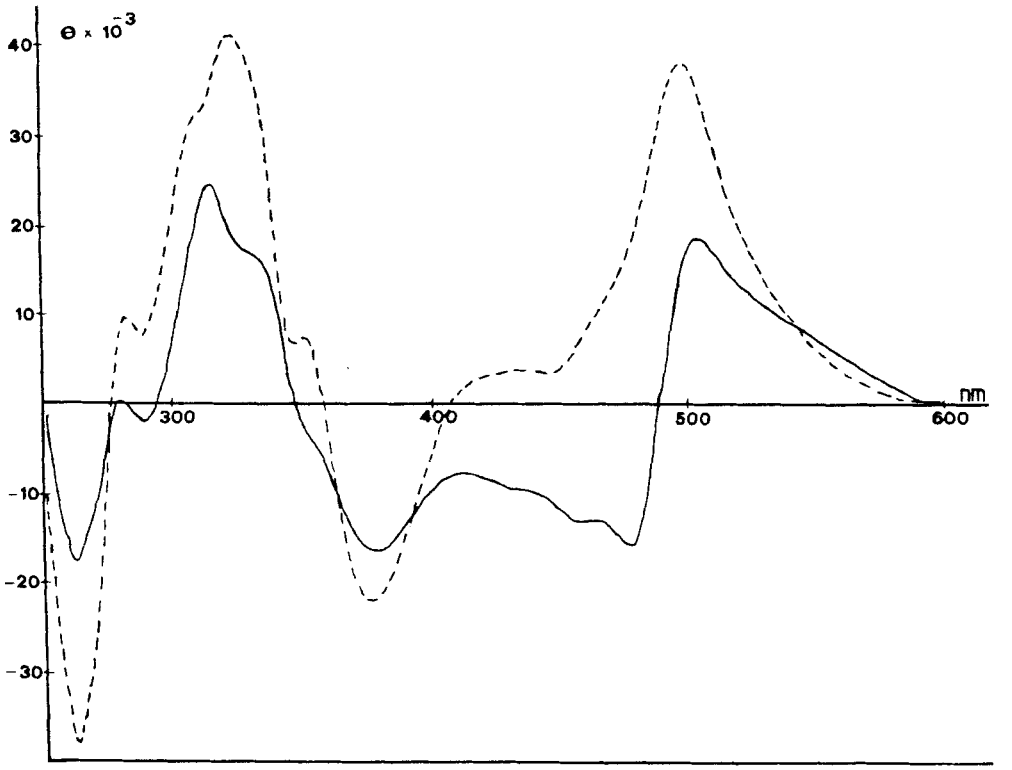


Fig. 3. *CD spectra of hexamethyl (5R,6R)-Coα,Coβ-dicyano-7-de(carboxymethyl)-5,6,7¹,7²-tetrahydro-5-hydroxy-7²-oxofuro[2,3-f]cobyrinate (2) (---) and heptamethyl (5R,6S)-Coα,Coβ-dicyano-5,6-dihydro-5-hydroxycobyrinate (3) (——)*

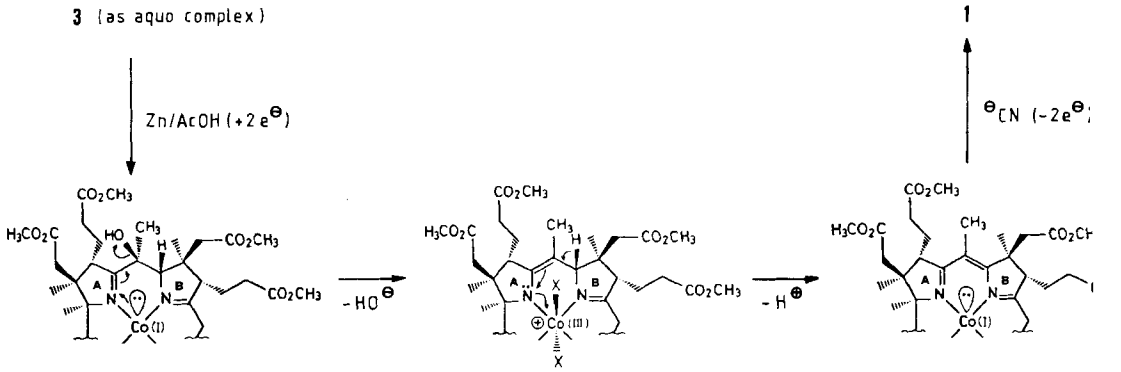
scopic data. The absolute configuration at C(6) of **3** was established to be *S* by ^{1}H-NMR NOE difference spectroscopy. In accordance with this assignment, the similarity of the CD spectra of **2** and **3** points out that both chromophores must have the same shape and, therefore, opposite absolute configurations at C(6), *(cf. Fig. 3)*.

The UV/VIS spectrum of **3** resembles that of the yellow corrinoid **2**; in the IR spectrum, however, the absorption band due to the carbonyl group of the five-membered lactone has disappeared. Seven (instead of six) CH_3O groups are present in the ^{1}H-NMR spectrum of **3**. Moreover, 2*s* at 4.88 (not present after H/D exchange) and 4.04 ppm reveal the presence of a OH group and H–C(6), respectively. As in **2**, CH_3–C(5) gives rise to a high-field-shifted *s* (at 1.64 ppm).

On irradiation of the proton of OH–C(5) in **3**, 6% enhancement of the intensity of the *s* at 4.04 ppm was detected. Reciprocally, on irradiation at 4.04 ppm, the intensity of the *s* at 4.88 ppm increased by 5.5% whereas the intensity of the signal at 1.64 ppm did not change significantly. Therefore, OH–C(5) which had been shown by X-ray analysis of **2** to be above the plane of the molecule and H–C(6) must be *cis* to each another. Moreover, the configuration at C(5) of **3** is confirmed by the observed 3% enhancement of the intensity of the signal of H–C(3) at 3.78 ppm, when the proton of OH–C(5) is irradiated. H–C(3) can be unequivocally assigned since for the signals of the same intensity and multiplicity corresponding to H–C(8) (2.56 ppm) and H–C(13) (2.88 ppm), clear NOE's were observed on irradiation of H–C(10) (5.02 ppm) and CH_3–C(15) (2.12 ppm), respectively.

Surprisingly enough, dehydratation of **3** in acidic medium did not occur. However, after transformation of **3** into the corresponding aquo complex, treatment with Zn/

Scheme 2. *Possible Mechanism for the Reductive Transformation of* **3** *into Heptamethyl Dicyanocobyrinate* (**1**)



AcOH as before led to heptamethyl dicyanocobyrinate (**1**). As Zn/AcOH is able to reduce the Co(III) ion in vitamin B_{12} to the corresponding Co(I) complex [24], it is likely that the lone pair of electrons in the latter participates in the elimination of the OH group at C(5) as depicted in *Scheme 2*. Reoxidation of the Co(I) ion occurs probably during workup in the presence of excess of CN^- ions (*cf.* [25]).

Since the discovery of the peculiar structure of the yellow corrinoid **2**, two new compounds with related structures have been isolated from natural sources. Thus, in 1979 *Koppenhagen et al.* reported that metal-free corrinoids isolated from the phototrophic bacteria *Rhodopseudomonas sphaeroides* and *Rh. capsulata* are irreversibly converted into yellow products under alkaline conditions, and the structure of the xanthocorrinoid obtained from hydrogenobyrinic acid *a,c*-diamide on treatment with aqueous 0.2N KOH was determined by X-ray analysis [10] [26] [27]. More recently, *Eschenmoser* and coworkers found that the bicyclic system at C(6),C(7) which is characteristic for xanthocorrinoids is also present in a Ni-containing porphinoid (so-called factor F 430 M) isolated by *Thauer*'s research group from *Methanobacterium thermoautotrophicum* cultures [28] [29].

Thus, the formation of structure **2** from **1**, surprising at the first glance, seems to obey to an inherent reactivity of the corrin chromophore, which is also present in peripherally reduced porphyrin derivatives. It is noteworthy, that intramolecular electrophilic attack of an acetamide side chain of coenzyme B_{12} on the vicinal C-atom of the corrin chromophore, as suggested to lead to the formation of the lactone ring of **2** (*cf.* below, *Scheme 5*), was postulated some years ago to proceed concomitantly with cleavage of the macrocycle in a speculative article concerning the biochemical catalytic action of vitamin-B_{12} coenzyme [30].

Any reaction mechanism which attempts to rationalize the transformation of **1** into **2** has to answer three questions: *i*) Why does the attack occur at C(5) stereospecifically? *ii*) Why is only the double bond between C(5) and C(6) involved in the reaction[8])? *iii*) Which role does the acetic-acid residue at C(7) play in the formation of xanthocorrinoids?

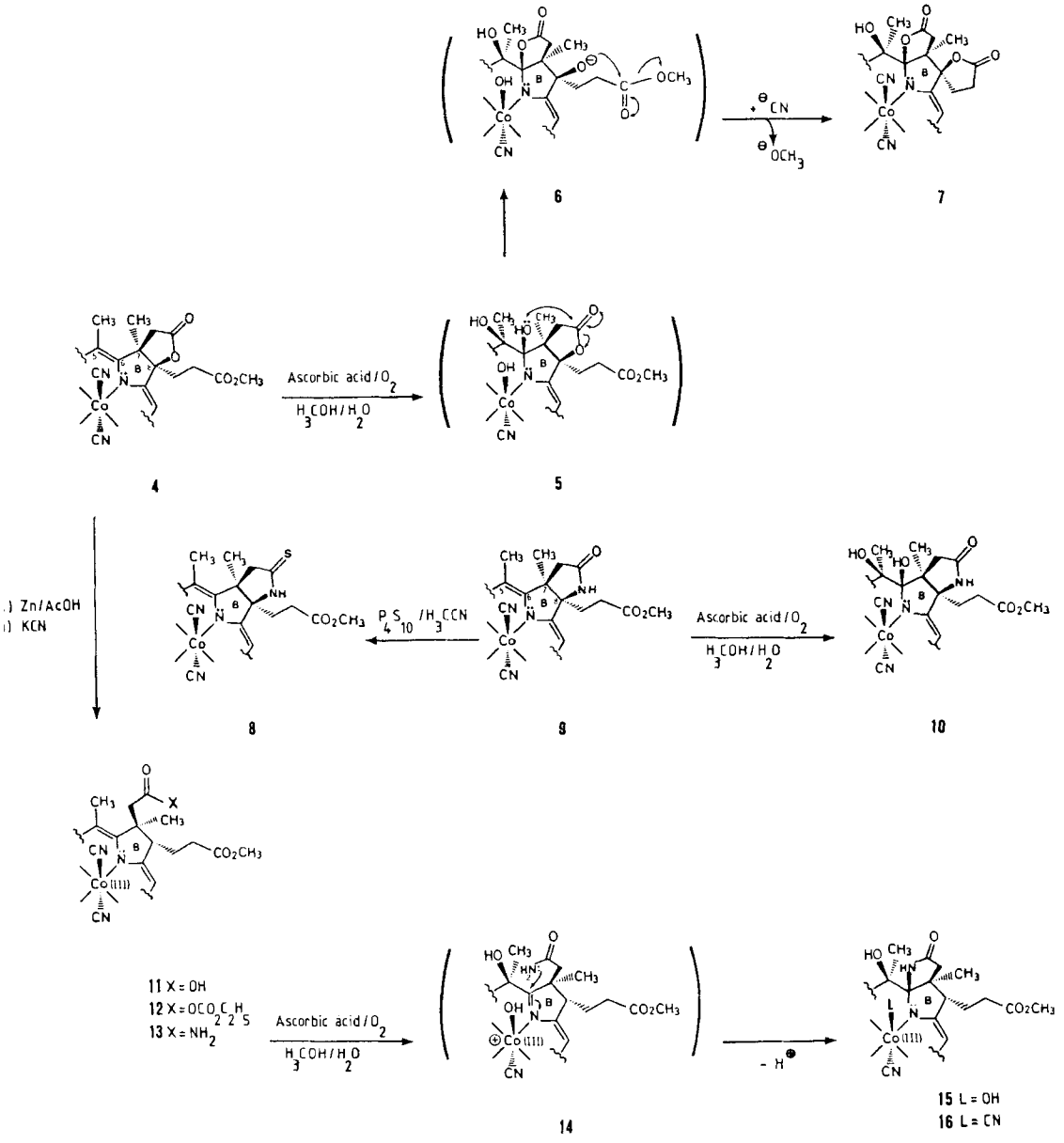
Although, to answer these questions, a knowledge of the nature of the reacting species would be important, no agreement could be found in the literature concerning the structure of the oxidizing agent present in systems which are considered models for enzymatic hydroxylations. Actually, the participation of OH radicals in *Fenton*'s system, which has been repeatedly invoked by several authors [31] [32] is reluctantly accepted by others because of their high and indiscriminative reactivity (*cf.* [33]). Nevertheless, the formation of yellow corrinoids by reaction of cyanocobalamin with OH radicals produced by radiolysis of H_2O has been reported [34].

Owing to the inherently complex nature of the *Udenfriend* system [35], no attempt was made in the scope of the present work to determine the actual structure of the species

[8]) As under optimum conditions 48% of unchanged **1** are recovered and a 30% yield of xanthocorrinoid **2** is obtained, reaction at C(10) and/or C(15) cannot be excluded. Until now, however, all our attempts to isolate isomers of **2** from the reaction mixture failed.

which bring about the hydroxylation of the corrin chromophore yielding xanthocorrinoids. Thus, the following considerations rather concern plausible structures of the intermediates involved in the formation of the latter. For the sake of simplicity, we consider H_2O_2, which is formed under *Udenfriend*'s conditions [15], to react with the corrin chromophore, although other nucleophiles such as HO_2 (perhydroxyl radical), O_2^-

Scheme 3



(superoxide ion), HO_2^- (hydroperoxide ion) or transition-metal complexes of these cannot be excluded.

To obtain more information on the structure of the precursor of **2**, the possibility of participation of the acetic-ester chain at C(7) in the stabilization of the final reaction product should be eliminated. Thus, we felt that the easiest way to realize this should be to use the known hexamethyl *Coα,Coβ*-dicyano-8-hydroxycobyrinate *c*-lactone (**4**)[6] [36], in which the *β*-substituent at C(7) is immobilized, as a substrate for *Udenfriend*'s reagent *(Scheme 3)*. Under the conditions described before, 16% of **4** was recovered and two yellow corrinoids were obtained. One of them proved to be identical with xanthocorrinoid **2** (13% yield). The spectroscopic data of the other product did not differ remarkably from those of **2**, the most manifest feature being the enhanced intensity of the IR absorption band at 1785 cm^{-1}. As in the case of **2**, the structure of this new xanthocorrinoid **7**[6]) was unequivocally elucidated by X-ray diffraction[9]).

Contrary to expectation, the lactone ring of **4** is opened during the reaction and two new lactone rings are formed. This result can be rationalized by the assumption that a diol **5**[6]) is generated first and subsequently transformed into the intermediate **6**[6]) by intramolecular transesterification of the *c*-lactone group *(Scheme 3)*. The released oxido group at C(8) in **6** might be prone to form a spirolactone by reaction with the propionic ester

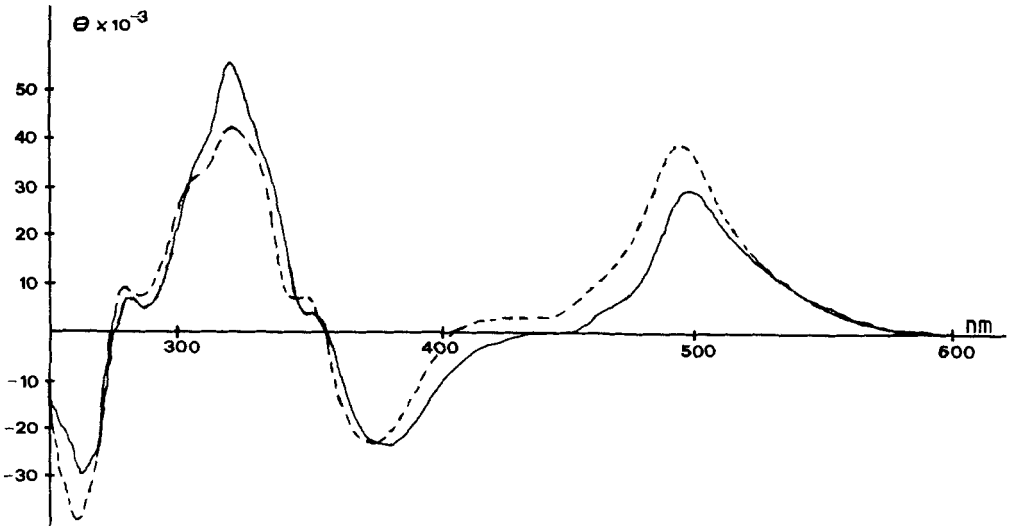


Fig. 4. *CD spectra of* **2** *(---) and hexamethyl (5*R*,6*R*)-8-amino-Coα,Coβ-dicyano-5,6-dihydro-5,6-dihydroxycobyrinate* c*-lactam (***10***) (——)*

[9]) Xanthocorrinoid **7** crystallises together with one molecule of H_2O in the orthorhombic space group $P2_12_12_1$ with a = 1564.4 (3), b = 2291.3 (5), c = 1543.2 (3) pm, Z = 4, $d_{calc.}$ = 1.38 g·cm^{-3}. The structure was solved by *Patterson* and difference syntheses and refined with the program SHELX-76 to the final reliability indices R = 0.130, R_w = 0.117 for 5046 independent reflections ($F_o^2 \geq 2.0\sigma(F_o^2)$, Cu$K\alpha$, $2\theta \leq 135°$). The terminal side chain methyl C-atoms, the C(3) side chain and the *d*-lactone ring atoms were refined isotropically, all other atoms anisotropically. H-Atoms were not included in the refinement. (Lists of atom-positional parameters, bond lengths, and bond angles may be requested from Prof. *W. S. Sheldrick*, University of Kaiserslautern.)

residue at the same position, thus explaining why no inversion of configuration occurs at this asymmetric C-atom.

To confirm the above results, hexamethyl 8-amino-*Coα,Coβ*-dicyanocobyrinate *c*-lactam (**9**)[6]), which was prepared in 74% overall yield from commercial cyanocobalamin by esterification of the known cobyrinic acid *c*-lactam [37] with $MeOH/H_2SO_4$, was treated with *Udenfriend*'s reagent under the conditions described before. All analytical data of the isolated xanthocorrinoid, (*ca.* 8% yield) agree with structure **10**[6]) (see *Exper. Part*). Particularly, the CD spectra of **2** and **10** are very similar *(cf. Fig. 4)*, thus pointing out that both chromophores must have the same shape and, therefore, the same configuration at C(6). As hydrolysis of the ester groups of **10** occurs to a large extent during the reaction, experiments directed to improve the yield of **10** are in progress [38].

Evidently, the lactam group of **9**, which is less easily hydrolizable than the lactone group of **4**, did not react with OH–C(6) after formation of the *cis*-diol **10**, so that the latter is the final product of the reaction. At this point, the replacement of the lactam group of **9** by a group more prone to a nucleophilic attack seemed appropriate in view of the possible formation of a lactone-spirolactam derivative analogous to **7**. Thus, hexamethyl dicyanocobyrinate *c*-thiolactam **8**[6]) was prepared from **9** by reaction with P_4S_{10} using the procedure given in [39], and **8** was reacted with O_2 in the presence of ascorbic acid as before. However, the reaction lead to a complex mixture of products which was not investigated in detail.

As the lactone ring of **4** can be opened by Zn/AcOH without affecting the ester groups present in the molecule [23], the monocarboxylic acid **11**[6]) was readily accessible free from regioisomers. Thus, the influence of specifically transformed *β*-substituent at C(7) on the formation of xanthocorrinoids under *Udenfriend*'s conditions could be investigated. The reluctance of the lactam ring of **10** to participate in an intramolecular reaction with OH–C(6) prompted us to investigate first the reactivity of hexamethyl *Coα,Coβ*-dicyanocobyrinate *c*-amide (**13**)[6]), which was synthesized in 93% overall yield from **11** by reaction with ethyl chloroformate and subsequent treatment of the resulting mixed anhydride **12**[6]) with dry NH_3.[10]). In contrast to lactam **9**, the monoamide **13** afforded, on reaction with O_2 in the presence of ascorbic acid, a yellow corrinoid with a 5-hydroxylactam structure **16**[6]) instead of a *cis*-diol corresponding to **10**. As, obviously, a *cis*-diol cannot be a precursor of **16**, the formation of the latter is rationalized in a straightforward

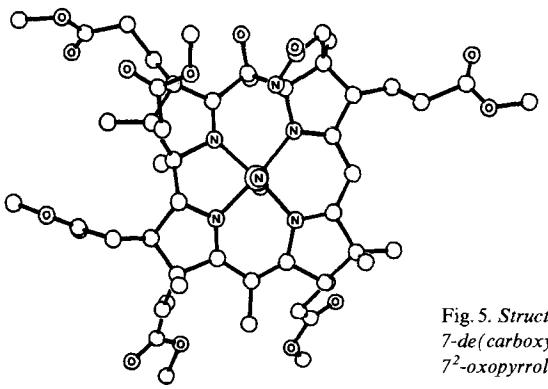
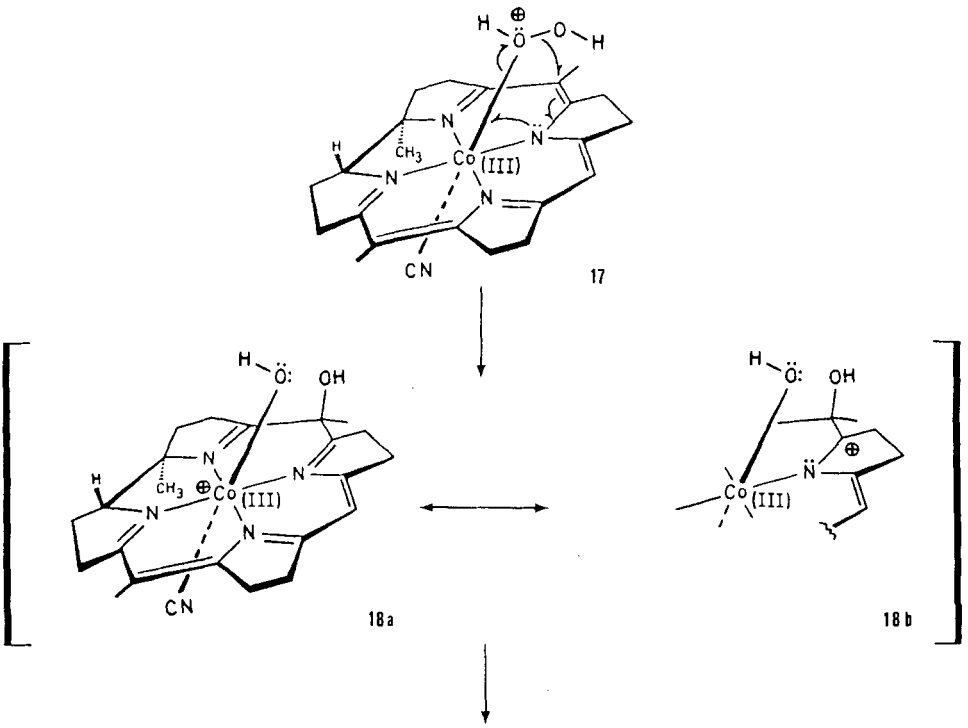


Fig. 5. *Structure of hexamethyl (5R,6S)-Coα,Coβ- dicyano-7-de(carboxymethyl)-5,6,7¹,7²-tetrahydro-5-hydroxy-7²-oxopyrrolo[2,3-f]cobyrinate (***16***)*

[10]) Hexamethyl *Coα,Coβ*-dicyanocobyrinate *c*-amide (**13**) has been obtained in 3% yield by partial methanolysis of cyanocobalamin [40].

Scheme 4. *Possible Participation of the Co-Ion in the Hydroxylation of the Corrin Chromophore under* Udenfriend's *Conditions*



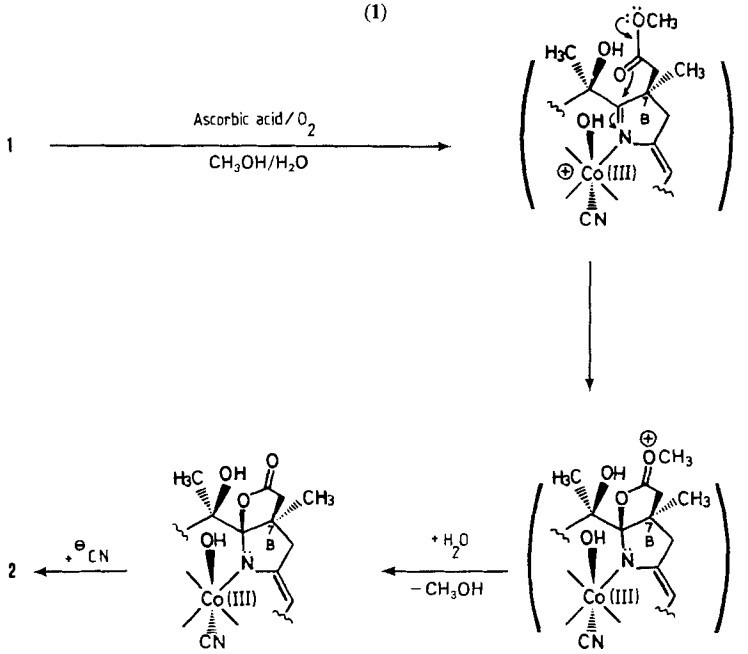
manner by assuming an intermediate **14** which leads to **15** by intramolecular nucleophilic attack of the amide N-atom at C(6). Exchange of the axial ligand on the Co-ion in the presence of CN^- ions during workup furnishes the product **16**, whose structure was established by X-ray diffraction[11]) (see *Fig. 5*).

The above results can be rationalized by the reaction mechanism depicted in *Scheme 4*[12]). Thus, the first step of the reaction of the corrin chromophore with *Udenfriend*'s reagent is suggested to be the replacement of the β-axial ligand on the Co-ion by H_2O_2 yielding **17**, followed by the rate-determining addition of a OH radical (or some equivalent) to C(5). The thus formed cationic intermediate **18**, in which the positive charge may be localized either at the Co(III) ion or at C(6), can either rearrange [11] or yield a *cis*-diol (*e.g.* **10**) by reaction with a molecule of H_2O. Depending on the structure of the substrate, however, intramolecular nucleophilic addition to the C(6)=N(22) bond may compete

[11]) Xanthocorrinoid **16** crystallises with one molecule of H_2O in the orthorhombic space group $P2_12_12_1$ with $a = 1581.0$ (3), $b = 2319.1$ (4), $c = 1508.2$ (3) pm, $Z = 4$, $d_{calc.} = 1.33$ g·cm^{-3}. The structure was solved by *Patterson* and difference syntheses and refined to $R = 0.088$, $R_w = 0.078$ for 4267 independent reflections ($F_o^2 \geq 3.0\sigma(F_o^2)$, Mo$K\alpha$, $2\theta \leq 50°$). With the exception of the water O-atom, all atoms were refined anisotropically. H-Atoms were not included in the refinement. (Lists of atom-positional parameters, bond lengths, and bond angles may be requested from Prof. *W. S. Sheldrick*, University of Kaiserslautern.)

[12]) For the sake of clarity, all peripheral substituents which do not participate in the reaction have been omitted.

Scheme 5. *Possible Mechanism of Formation of Xanthocorrinoid* **2** *from Heptamethyl Coα,Coβ-Dicyanocobyrinate* (**1**)



with addition of H_2O (as in the case of **16**), or the *cis*-diol may give rise to further intramolecular reactions such as transesterification of the *c*-acetic-ester substituent at C(7) yielding **2** or the spirolactone **7**.

Actually, intermediate **18** may be transformed into product **2** either *via* a *cis*-diol corresponding to **5** or directly by intramolecular nucleophilic attack of the carbonyl group of the acetic-ester chain at C(7) *(cf. Scheme 5)*. Unfortunately, experiments with $^{18}O_2$ in ^{18}O-enriched solvents, which were carried out in order to elucidate the origin of the O-atom bound to C(6) in the product **2** by the isotope effect on the chemical shift of the corresponding ^{13}C-NMR signal (*cf.* [41]), were not conclusive [42].

The reaction mechanism depicted in *Scheme 4* does not only rationalize plausibly the formation of different xanthocorrinoids as a function of the structure of the substrate, but it accounts also for the regio- and stereospecificity of the reaction. Actually, the lack of reactivity at C(10), which is usually prone to reaction with *electrophiles* [3a], may be explained by the higher double-bond character of the C(5),C(6) bond [43]. On this ground alone, however, a preference for the latter over the C(14),C(15) bond is not obvious. Provided that the complexed Co-ion participates in the formation of the hydroxylating species as depicted in *Scheme 4*, the regioselectivity of the reaction might be conditioned by the geometry of the complex in which the axial ligand situated above the plane of the molecule is closer to C(5) than to C(15) (*cf.* [44]). However, the fact that in most of the compounds investigated so far the substituent at C(7) participates in the stabilization of the final product prompted us to investigate the behaviour of a substrate in which the substituents at this position are completely inert under *Udenfriend*'s conditions [11].

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Experimental Part

General. Prep. TLC: plates (20 × 20 or 100 × 20 cm) precoated with silica gel F_{254} or silica gel *H* (both from *E. Merck*, D-6100 Darmstadt) containing 1% of KCN; CH_2Cl_2/MeOH 97:3 as eluent. M.p.'s: *Kofler* hot stage melting point apparatus (*THERMOVAR, C. Reichert AG*, Vienna); not corrected. IR: *Perkin-Elmer-IR-521* and *-599* spectrometers; $CHCl_3$ solns. unless otherwise specified; frequencies in cm^{-1}. UV/VIS: *Leitz-Unicam-SP-800-B* and *Perkin-Elmer-320* spectrometers; MeOH solns. containing 0.01% of KCN unless otherwise specified; λ_{max} ($\log\varepsilon$) in nm. CD: *Jobin-Yvon-Auto-Dichrograph-Mark-V*[13]) and *Roussel-Jouan-Dichrograph-II* instruments; MeOH solns.; band amplitudes are given as specific ellipticity (θ) and wavelengths (λ) in nm. ^{1}H- and ^{13}C-NMR: $CDCl_3$ solns.; *Varian- XL-100, Bruker- AM-360*, and *Bruker-WM-400* instruments; chemical shifts in ppm are referenced to internal TMS and $CDCl_3$ (77.05 ppm), resp., *J* values in Hz. NMR spectra on the *Bruker*-AM-360 instrument were recorded by M. *E. Trieschmann*. MS: *Vacuum Generators Micromass 7070 E* instrument equipped with a data system *DS 11-250*. At an acceleration voltage of 6 kV using the FAB-ionization technique with Ar at 8 kV. Elemental analyses were performed by *I. Beetz*, Microanalytical Laboratories, D-8640 Kronach, and by Mr. *F. Nydegger*.

Heptamethyl Coα,Coβ-*Dicyanocobyrinate* (**1**) was prepared from commercial cyanocobalamin (*E. Merck*, D-6100 Darmstadt) according to *Keese*'s method as reported in [45]. *Heptamethyl amminocyanocobyrinate* was obtained in 64% yield by shaking 5% aq. NH_4OH with a CH_2Cl_2 soln. of the corresponding aquocyanocomplex, whose preparation is described in [45]. *Heptamethyl cyanocob(II)yrinate* was obtained from the latter by catalytical reduction on Pd/C. Owing to its sensitivity to air it was reacted with ascorbic acid without previous isolation. The much more stable *heptamethyl iodocob(II)yrinate* was prepared by reduction of the corresponding diiodocob(III)yrinate with sodium thiosulfate (*cf.* [45]).

*Hexamethyl (5*R,6R*)*-Coα,Coβ-*Dicyano-7-de(carboxymethyl)-5,6,7*^{1}*,7*^{2}*-tetrahydro-5-hydroxy-7*^{2}*-oxofuro-[2,3-f]cobyrinate;* (**2**). i) *General Procedure.* To a soln. of **1** (100 mg), ascorbic acid (400 mg), and $NaHCO_3$ (210 mg) in MeOH (10 ml) containing 16 ml of phosphate buffer (pH 7.2), an aq. soln. of EDTA (0.01M, 2ml) was added, and a gentle stream of O_2 was passed into the mixture for 3 h at 65°. After cooling to r.t., 20 ml of sat. aq. NaCl soln. were added, and the mixture was extracted repeatedly with CH_2Cl_2 until the aq. phase became colorless. The combined org. phases were filtered through a cotton plug and evaporated. From the residue, **2** and remaining **1** were isolated by prep. TLC.

ii) *Oxidation of* **1** *with* H_2O_2. To a stirred soln. of **1** (20 mg) in MeOH (5.5 ml) containing 11 ml of phosphate buffer (pH 7.2), 3 ml of a dil. H_2O_2/MeOH soln. (prepared from 3.3 ml of 30% aq. H_2O_2 and 97 ml of MeOH) were added dropwise within 45 min at 40°. The mixture was cooled down, diluted with H_2O and extracted repeatedly with CH_2Cl_2, until the org. layer stayed colorless. Isolation of **2** (6 mg, 30%) as above. On crystallization of **2** from benzene/hexane and MeOH/Et_2O, yellow needles, m.p. 176–178° and 196–197°, resp. were obtained. IR ($CHCl_3$): 3420, 2990, 2960, 2125, 1785, 1735, 1600, 1580, 1530, 1500, 1440, 1410, 1310. UV/VIS: 488 (4.00), 469 (3.99), 321 (4.02), 269 (sh), 220 (4.73). CD ($7.80\cdot10^{-5}$M): 496 (38053), 444 (3171), 430 (3382), 406 (0), 375 (−22409), 358 (0), 351 (6976), 347 (6342), 323 (40801), 311 (sh, 32133), 288 (7188), 281 (9302), 276 (0), 262 (−38053). ^{1}H-NMR (100 MHz): 5.39 (*s*, exchangeable for D, OH); 5.04 (*s*, H–C(10)); 4.22 (*d*, *J* = 10, H–C(19)); 3.75, 3.74 (6 H), 3.70, 3.67, 3.64 (5*s*, 6 CH_3O); 2.17 (*s*, CH_3–C(15)); 1.85 (*s*, CH_3–C(5)); 1.44 (6 H), 1.29 (6 H), 1.22, 1.13 (4*s*, 6 CH_3). ^{13}C-NMR (25.16 MHz): 191.8 (*s*, C(4)); 176.8, 175.6, 175.0, 174.4, 174.0, 172.9, 172.8 (2 C), 172.2, 172.0 (9*s*, 6 $COOCH_3$, C(7^2), C(9), C(11), C(16)); 164.6 (*s*, C(14)); 134.1, 129.8 (2 br. *s*, 2 C≡N); 112.2 (*s*, C(6)); 100.8 (*s*, C(15)); 85.6 (*d*, C(10)); 84.8 (*s*, C(1)); 78.9 (*s*, C(5)); 76.1 (*d*, C(19)); 59.0 (*d*, C(3)); 58.5 (*s*, C(17)); 56.0 (*d*, C(8)); 53.7 (*d*, C(13)); 52.4 (2C), 52.0, 51.8 (2C), 51.7 (4 *q*, 6 CH_3O); 50.8 (*s*, C(7)); 46.8, 45.5 (2*s*, C(2), C(12)); 45.4 (*t* C(7^1)); 40.7 (*t*, C(2^1)); 40.0 (*d*, C(18)); 33.0, 32.5, 32.3, 31.7, 31.2, 30.0, 25.8, 23.2, 22.4 (9*t*, 9 CH_2); 30.7 (*q* β-CH_3–C(12)); 23.2 (*q*, CH_3–C(5)); 15.0 (*q*, CH_3–C(5)); 21.7, 20.1, 18.4, 17.3, 16.1 (5*q*, 5 CH_3). Anal. calc. for $C_{53}H_{71}CoN_6O_{15}$ (1091.1): C 58.34, H 6.56, N 7.70, O 22.00; found: C 58.23, H 6.56, N 7.58, O 22.05.

*Heptamethyl (5*R,6S*)*-Coα,Coβ-*Dicyano-5,6-dihydro-5-hydroxycobyrinate* (**3**). A soln. of **2** (30 mg) in dry CH_2Cl_2 (15 ml) containing Zn dust[14]) (600 mg) was deoxygenated by repeated freezing under Ar and thawing under high vacuum. Thereafter, the mixture was refluxed gently under Ar, and 1.2 ml of AcOH were injected through a rubber septum within 2 h. After 135 min, the resultant olive-green suspension was diluted with 30 ml of CH_2Cl_2 and filtered through cellulose. A soln. of KCN (1.5 g) in H_2O (30 ml) was added to the filtrate, and the mixture was stirred for 20 min in the presence of air. The resultant reddish-violet soln. was separated and successively washed

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[13]) Granted by the *Swiss National Science Foundation*.

[14]) Purified according to [46].

once with sat. aq. NaCl soln., 10% AcOH, and H_2O. The org. layer was filtered through a cotton plug, the solvent evaporated, and the residue dried by azeotropic distillation with benzene. The crude product was dissolved in CH_2Cl_2 (6 ml), and the soln. was deoxygenated as before. Thereupon, 0.5N CH_2N_2/Et_2O (0.3 ml) was added, and the mixture was allowed to stand in the dark for 2 h at r.t. After evaporation of the solvent, **1** (17 mg, 57%) and yellow **3** (8mg, 27%) were isolated from the residue by prep. TLC. Recrystallization from benzene/hexane gave pure **3**, m.p. 201–202° (dec.). IR ($CHCl_3$): 3420, 3120, 2980, 2950, 2920, 2850, 2110, 1730, 1585, 1498, 1437, 1410, 1375. UV/VIS: 482 (3.97), 464 (sh), 319.5 (4.01), 266 (3.79), 216 (4.67). CD ($9.73 \cdot 10^{-5}$M): 590 (0), 501 (18488), 486 (0), 474 (−16113) 455 (−13569), 410 (−8141), 377 (−16961), 347 (0), 330 (16792), 314 (24424), 294 (0), 289 (−2541), 279 (0), 262 (−19336). ^{1}H-NMR (360 MHz): 5.04 (*s*, H–C(10)); 4.82 (*s*, exchangeable for D, OH); 4.06 (*s*, H–C(6)); 3.98 (*d*, J = 10, H–C(19)); 3.76 (6 H), 3.74, 3.73, 3.72, 3.71, 3.66 (6*s*, 7 CH_3O); 2.13 (*s*, CH_3–C(15)); 1.64 (*s*, CH_3–C(5)); 1.41, 1.33, 1.26 (6 H), 1.22, 1.11 (5*s*, 6 CH_3).

Transformation of **3** into **1**. A soln. of **3** (10 mg) in CH_2Cl_2 (4 ml) was shaken for 3 min with 30% aq. perchloric acid (1 ml). The org. layer was separated, washed twice with H_2O, and filtered through a cotton plug. After evaporation of the solvent, the residue was dissolved in MeOH (2 ml) under N_2, and Zn dust[14]) (80 mg) was added at once. To the stirred suspension, AcOH (0.5 ml) was injected through a rubber septum. After 30 min, the Zn was filtered off, and KCN (40 mg) was added to the filtrate. After evaporation, prep. TLC of the residue afforded **1** whose mixed m.p. (188–190°) with an authentic sample showed no depression.

Spiro{pentamethyl (5R,6R,7S,8R)-Coα,Coβ-dicyano-8-de(carboxyethyl)-7-de(carboxymethyl)-5,6,7^1,7^2-tetrahydro-5-hydroxy-7^2-oxofuro[2,3-f]cobyrinate-8,2'-tetrahydrofuran-5'-one} (**7**). To a soln. of hexamethyl *Coα,Coβ*-dicyanocobyrinate *c*-lactone [35] (100 mg), ascorbic acid (400 mg), and $KHCO_3$ (238 mg) in 15 ml of MeOH containing 16 ml of phosphate buffer (pH 7.2), an aq. soln. of EDTA (0.01M, 2 ml) was added, and a gentle stream of O_2 was passed into the mixture for 3 h at 65°. After cooling to r.t., 40 ml of sat. aq. NaCl soln. were added, and the mixture was extracted repeatedly with CH_2Cl_2 until the org. phase stayed colorless. The combined org. phases were dried by filtration through cotton, and the solvent was evaporated. The product was purified by prep. TLC using CH_2Cl_2/MeOH 93:3 containing 0.1% of KCN. Recrystallization from Et_2O/MeOH gave **7** (17 mg, 17%) as dark-brown needles: m.p. 169° (dec.). IR: 3420, 2990, 2960, 2125, 1785, 1735, 1600, 1575, 1545, 1500, 1440, 1410, 1375. UV/VIS: 500 (3.94), 477 (3.95), 3.24 (4.03), 274 (sh), 219 (4.58). ^{1}H-NMR (100 MHz): 5.01 (*s*, H–C(10)); 4.40 (*d*, J = 10, H–C(19)); 3.75, 3.72 (6 H), 3.70, 3.64 (4*s*, 5 CH_3O); 2.20 (*s*, CH_3–C(15)); 1.72 (*s*, CH_3–C(5)); 1.55, 1.40, 1.26 (9 H), 1.14 (4*s*, 6 CH_3). ^{13}C-NMR (25.16 MHz): 192.3 (*s*, C(4)); 177.0, 175.7 (2*s*, C(11), C(16)); 174.7, 173.8 (2 C), 172.8, 172.4, 171.9 (2 C), 171.4 (6*s*, 5 *C*OOCH$_3$, C(7^2), C(8^3) C(9)); 163.6 (*s*, C(14)); 132.5, 128.3 (2*s*, 2 C≡N); 107.8 (*s*, C(6)); 101.4 (*s*, C(15)); 93.1 (*s*, C(8)); 84.7 (*s*, C(1)); 82.5 (*d*, C(10)); 77.5 (*s*, C(5)); 76.2 (*d*, C(19)); 59.5 (*d*, C(3)); 58.7 (*s*, C(17)); 53.7 (*d*, C(13)); 53.3 (*s*, C(7)); 52.4, 52.0, 51.9 (2 C), 51.7 (4*q*, 5 CH_3O); 46.8, 46.0 (2*s*, C(2), C(12)); 42.6 (*t*, C(7^1)); 41.1 (*t*, C(2^1)); 39.7 (*d*, C(18)); 32.9, 32.4, 31.3, 30.8, 29.7 (2 C), 28.6, 25.6, 23.6 (8*t*, 9 CH_2); 31.6 (*q*, *β*-*C*H$_3$–C(12)); 21.8, 21.4 (2*q*, *C*H$_3$–C(1), *C*H$_3$–C(5)); 19.7 (*q*, *α*-*C*H$_3$–C(12)); 17.9, 17.5, 16.5 (3*q*, *C*H$_3$–C(2), *C*H$_3$–C(7), *C*H$_3$–C(17)); 15.3 (*q*, *C*H$_3$–C(15)). Anal. calc. for $C_{52}H_{67}CoN_6O_{15}$ (1075.1): C 58.10, H 6.28, N 7.82, O 22.32, CH_3O 14.43; found: C 57.92, H 6.13, N 7.12, O 22.74, CH_3O 14.60.

Hexamethyl 8-Amino-Coα,Coβ-dicyanocobyrinate c-Thiolactam (**8**). To a soln. of **9** (0.535 g; see below) in MeCN (20 ml), $NaHCO_3$ (0.21 g) and P_4S_{10} (1.11 g) were added, and the mixture was stirred under N_2 at r.t. for 8 h. The mixture was then poured into H_2O (500 ml) and repeatedly extracted with CH_2Cl_2 (2 × 100 ml). The combined extracts were washed with 1% aq. KCN (2 × 500 ml), filtered, and evaporated under reduced pressure. The product was isolated by column chromatography on silica gel containing 0.5% of KCN using a AcOEt/AcOMe 1:1. Recrystallisation from CH_2Cl_2/pentane yielded 0.370 g (68%) of **8**, m.p. 197–200° (dec.). IR (CH_2Cl_2): 3380*w*, 2950*m*, 2120*w*, 1735*s*, 1580*m*, 1510*s*, 1485*m*, 1440*m*, 1400*m*, 1370*m*, 1210*s*, 1170*s*. UV/VIS: 583 (3.98), 542 (3.92), 504 (3.74), 366 (4.45), 274 (4.37). CD ($5.00 \cdot 10^{-5}$M): 581 (−2640), 519 (0), 421 (29040), 372 (77880), 353 (0), 342 (−26400), 305 (−79200), 281 (−85800), 274 (0), 265 (59400). ^{1}H-NMR (360.13 MHz): 9.51 (*s*, NH); 5.61 (*s*, H–C(10)); 3.76, 3.73, 3.69, 3.68, 3.64, 3.62 (each *s*, 6 CH_3O); 2.25, 2.19, 1.59, 1.52, 1.36 (6 H), 1.26, 1.19 (each *s*, 8 CH_3). ^{13}C-NMR (90.56 MHz): 202.01 (*s*, C(7^2); 178.12, 176.08, 175.26, 173.68, 173.54, 172.79, 172.71, 171.79, 171.66, 166.33, 163.19, 160.44, (12*s*, 6 *C*OOCH$_3$ C(4), C(6), C(9), C(11), C(14), C(16)); 103.56 (*s*, 2 C, C(5), C(15)); 130.00 (*s*, 2 C≡N); 88.33 (*d*, C(10)); 82.68 (*s*, C(1)); 81.01 (*s*, C(8)); 75.19 (*d*, C(19)); 58.39 (*s*, C(17)); 56.63 (*d*, C(3)); 54.94 (*s*, C(7)); 53.69 (*d*, C(13)); 47.58, 45.70 (2*s*, C(2), C(12)); 39.30 (*d*, C(18)); 52.39, 52.04, 51.84 (3 C), 51.60 (4*q*, 6 CH_3O); 56.63, 41.00, 33.65, 32.65, 31.72, 30.69, 29.74, 29.50, 29.01, 25.66, 24.94 (11*t*, 11 CH_2); 30.89 (*q*, *β*-*C*H$_3$–C(12)); 22.06, 19.68 (2 C), 18.55, 16.88, 16.82, 15.29 (6 *q*, 7 CH_3). FAB-MS (glycerol): 1037 (100, $M + 2)^+ - 2$ CN), 1008 (8), 964 (37), 936 (6), 921 (5), 878 (12), 862 (7). Anal. calc. for $C_{53}H_{70}CoN_7O_{17}S$ (1088.2): C 58.50, H 6.48, N 9.01; found: C 58.06, H 6.60, N 8.79.

Hexamethyl 8-Amino-Coα,Coβ-dicyanocobyrinate c-Lactam (**9**). A soln. of cyanocobalamin (5 g, *E. Merck*) and NaOH (75.0 g) in H_2O (250 ml) was heated for 8 h under reflux. After cooling to r.t., HCl (35%, 175 ml) was

added, and the mixture was repeatedly extracted with phenol (3 × 50 g). The combined phenolic phases were washed with H_2O (50 ml) which was subsequently reextracted with phenol (2 × 10 g). Aq. NH_3 (0.2M, 300 ml), and Et_2O (400 ml) were added to the combined org. phases, the aq. layer was separated and washed with Et_2O (2 × 200 ml), evaporated and dried at 0.01 Torr over P_4O_{10} for 12 h. The residue was refluxed with 2% H_2SO_4 in MeOH (150 ml) for 24 h. To the cooled mixture, H_2O (750 ml), $NaHCO_3$ (10 g), and KCN (0.5 g) were added, followed by extraction with CH_2Cl_2 (2 × 250 ml). The extracts were dried over Na_2SO_4 and evaporated. The product was isolated by column chromatography on silica gel containing 0.5% of KCN, using CH_2Cl_2/MeOH 97:3. Recrystallization from MeOH yielded 2.91 g (74%) of **9**, m.p. 248 – 250° (dec.). IR (CH_2Cl_2): 3420*w*, 2950*m*, 2120*w*, 1735*s*, 1710*s*, 1580*s*, 1505*s*, 1435*m*, 1370*m*, 1205*s*, 1175*s*, 1155*s*. UV/VIS: 582 (4.01), 542 (3.93), 506 (3.74), 365 (4.46), 314 (3.96), 277 (3.98). CD ($7.13 \cdot 10^{-5}$M): 581 (–10645), 543 (–8331), 486 (0), 421 (29621), 395 (45358), 366 (0), 347 (–26844), 328 (0), 324 (5091), 318 (0), 306 (–10645), 281 (–24067), 255 (–22216), 254 (–17588). ^{1}H-NMR (400 MHz): 7.71 (*s*, NH); 5.55 (*s*, H–C(10)); 3.86 (*dd*, *J* = 9.1, 1.6, H–C(3)); 3.82 (*d*, *J* = 10.6, H–C(19)); 3.76, 3.73, 3.70, 3.68, 3.63, 3.61 (6*s*, 6 CH_3O); 3.02 (*dd*, *J* = 6.6, 4.3, H–C(13)); 2.84 (*dt*, *J* = 10.6, *ca.* 6.5, H–C(18)); 2.24, 2.20, 1.62, 1.53, 1.36 (6 H), 1.26, 1.19 (each *s*, 8 CH_3). ^{13}C-NMR (100.61 MHz): 177.74, 175.58, 174.94, 173.93, 173.38, 172.89, 172.51, 172.40, 171.53, 171.39, 169.02 (11*s*, 6 *C*OOCH$_2$, C(7^2), C(4), C(9), C(11), C(16)); 163.14, 161.31 (2*s*, C(6), C(14)); 130.0, 129.5 (2*s*, 2 C≡N); 102.92, 102.51 (2*s*, C(5), C(15)); 88.19 (*d*, C(10)); 82.30 (*s*, C(1)); 74.75 (*d*, C(19)); 73.40 (*s*, C(8)); 57.94 (*s*, C(17)); 56.29 (*d*, C(3)); 53.36 (*d*, C(13)); 51.98, 51.44 (3 C), 51.38, 51.15 (4*q*, 6 CH_3O); 50.81 (*s*, C(7)); 47.07 (*s*, C(12)); 45.37 (*s*, C(2)); 44.78 (*t*, C(7^1)); 40.73 (*t*, C(2^1)); 39.00 (*d*, C(18)); 33.32, 32.30, 31.36, 30.35, 30.14, 29.36, 28.69, 25.32, 24.63 (9*t*, 11 CH_2); 30.65 (*q*, *β*-*C*H$_3$–C(12)); 21.68 (*q*, *C*H$_3$–C(1)); 19.65, 19.31 (2*q*, *α*-*C*H$_3$–C(12), *C*H$_3$–C(7)); 18.06 (*q*, *C*H$_3$–C(17)); 16.52 (*q*, *C*H$_3$–C(2)); 16.48 (*q*, *C*H$_3$–C(5)); 14.86 (*q*, *C*H$_3$–C(15)). FAB-MS (glycerol): 1046 (23, $(M+1)^+ - CN$), 1019 (91, $(M+1)^+ - HCN - CN$), 917 (14), 859 (17), 193 (23), 55 (100). Anal. calc. for $C_{53}H_{70}CoN_7O_{13} \cdot H_2O$ (1090.1): C 58.39, H 6.66, N 8.99; found: C 58.30, H 6.60, N 8.98.

*Hexamethyl (5*R*,6*R*)-8-Amino-*Co*α*,Co*β*-dicyano-5,6-dihydro-5,6-dihydroxycobyrinate* c-*Lactam* (**10**). To a soln. of **9** (1.073 g) in MeOH (150 ml) containing 160 ml of phosphate buffer (pH = 7.2), ascorbic acid (4.0 g), $KHCO_3$ (2.38 g), and EDTA (4.7 mg) were added, and a gentle stream of air was passed through the mixture for 4 h at 65°. After cooling to r.t., 300 ml of sat. aq. NaCl were added, and the mixture was extracted with CH_2Cl_2 (3 × 100 ml). The combined extracts were evaporated *in vacuo*. The product **10** (0.071 g, 8%) and remaining **9** (0.239 g) were isolated by prep. TLC using acetone/AcOEt 1:1 containing 0.1% of KCN. Recrystallisation from MeOH/Et_2O gave pure **10**, m.p. 208–210°. IR (CH_2Cl_2): 3400*w*, 2955*m*, 2120*w*, 1735*s*, 1710*s*, 1580*w*, 1540*w*, 1440*w*, 1410*m*, 1385*m*, 1200*s*, 1175*s*. UV/VIS: 487 (4.06), 470 (4.04), 320 (4.05). CD ($5 \cdot 10^{-5}$M): 498 (29040), 444 (0), 379 (–23100), 358 (0), 353 (3960), 350 (3300), 322 (54780), 290 (4620), 282 (6600), 276 (0), 263 (–28710). ^{1}H-NMR (360.13 MHz): 6.21 (*s*, exchangeable by D, NH); 5.12 (*s*, H–C(10)); 4.51 (*s*, exchangeable by D, OH); 4.08 (*d*, *J* = 10.4, H–C(19)); 3.96 (*s*, exchangeable by D, OH); 3.74, 3.71, 3.70 (6 H), 3.68, 3.64 (5*s*, 6 CH_3O); 2.17 (*s*, CH_3–C(15)); 1.72, 1.46, 1.42, 1.29 (6 H), 1.21, 1.11 (6*s*, 7 CH_3). ^{13}C-NMR (100.61 MHz): 194.27 (*s*, C(4)); 176.73, 175.40, 174.99, 173.90, 173.49, 173.43, 172.88, 172.85, 171.71, 171.50 (10*s*, 6 *C*OOCH$_3$, C(7^2), C(9), C(11), C(16)); 164.67 (*s*, C(14)); 134.1, 131.6 (2*s*, 2 C≡N)); 102.50, 101.37 (2*s*, C(6), C(15)); 84.61 (*s*, C(1)); 84.37 (*d*, C(10)); 81.88 (*s*, C(5)); 76.15 (*d*, C(9)); 73.59 (*s*, C(8)); 60.26 (*d*, C(3)); 58.48 (*s*, C(17)); 55.15 (*s*, C(7)); 53.82 (*d*, C(13)); 52.41, 52.15, 51.87, 51.83, 51.65 (5*q*, 6 CH_3O); 47.03, 44.73 (2*s*, C(2), C(12)); 43.52, 41.05 (2*t*, C(2^1), C(7^1)); 40.02 (*d*, C(18)); 30.51 (*q*, *β*-*C*H$_3$–C(12)); 33.01, 32.67, 31.76, 31.01, 29.91, 29.31, 28.45, 25.76, 23.89 (9*t*, 9 CH_2); 23.05, 21.30, 20.01, 19.46, 18.61, 16.43, 14.93 (7*q*, 7 CH_3). FAB-MS ((2-nitro-phenyl)octylether): 1105 (39, M^+), 1079 (100, $M^+ - CN$), 1065 (40), 1053 (9, $M^+ - 2\,CN$).

Hexamethyl Co*α*,Co*β*-*Dicyanocobyrinate* c-*Amide* (**13**). To 50 ml of a soln. of **11** [23] (240 mg) in $CHCl_3$, 0.08 ml of Et_3N and 0.06 ml of ethyl chloroformate were added at –5°, and the mixture was stirred for 1 h. The resulting soln. of the mixed anhydride **12** was warmed to r.t. and a gentle stream of dry NH_3 was bubbled through for 5 min with stirring. After 1 h, the mixture was washed once with 3% aq. HCl, once with dist. H_2O, once with 2% aq. KCN and finally again with dist. H_2O. The solvent was removed *in vacuo* and the violet residue purified by prep. TLC using CH_2Cl_2/MeOH 95:5 containing 0.1% of KCN. Recrystallization from cyclohexane/AcOMe yielded 0.225 g (93%) of **13**, m.p. 136–138°. IR ($CHCl_3$): 3040*m*, 2980*m*, 2960*m*, 2125*w*, 1730*s*, 1675*m*, 1585*m*, 1545*m*, 1495*m*, 1440*m*, 1420*m*. UV/VIS: 582 (4.02), 542 (3.92), 511 (sh, 3.71), 418 (3.35), 368 (4.46), 353 (sh, 4.14), 311 (3.95), 277 (4.02). CD ($4.89 \cdot 10^{-5}$M): 577 (–10588), 554.5 (–2765), 534 (–6069), 516 (–3574), 499 (–4855), 461 (0), 426 (37765), 417.5 (34730), 395.5 (64740), 373 (0), 366.5 (–17736), 361 (–15039), 346 (–32235), 327 (–19422), 320 (–11195), 310 (–30077), 286 (0), 278 (–2698), 272 (–1821), 253 (–37428), 245 (–28998), 236 (0). ^{1}H-NMR (360.13 MHz): 6.98, 5.25 (2*s*, 2 NH); 5.54 (*s*, H–C(10)); 3.77, 3.71 (6 H), 3.70, 3.68, 3.64 (5*s*, 6 CH_3O); 2.23, 2.17 (2*s*, CH_3–C(5), CH_3–C(15)); 1.80, 1.51, 1.37 (6 H), 1.26, 1.21 (5*s*, 6 CH_3). ^{13}C-NMR (90.56 MHz): 175.94, 175.79, 175.48 (3 *s*, C(4), C(11), C(16)); 173.77, 173.52, 172.81, 172.45, 172.09, 171.57, 171.52, 171.11 (8*s*, 6 *C*OOCH$_3$,

C(7^2), C(9)); 163.56, 160.99 (2*s*, C(6), C(14)); 106.89 (*s*, C(5)); 102.31 (*s*, C(15)); 91.29 (*d*, C(10)); 82.63 (*s*, C(1)); 74.71 (*d*, C(19)); 58.50 (*s*, C(17)); 58.42 (*d*, C(8)); 56.77 (*d*, C(3)); 53.58 (*d*, C(13)); 52.41, 51.99, 51.84 (2 C), 51.62 (2 C) (4*q*, 6 CH_3O); 51.20 (*s*, C(7)); 46.99, 46.22 (2*s*, C(2), C(12)); 46.37 (*t*, C(7^1)); 41.84 (*t*, C(2^1)); 39.19 (*d*, C(18)); 33.76, 32.46, 31.75, 30.86, 30.82, 29.67 (6*t*, 6 CH_2); 25.86, 25.77, 24.87 (3*t*, C(3^1), C(8^1), C(13^1)); 31.38 (*q*, β-CH_3–C(12)); 22.05 (*q*, CH_3–C(1)); 19.80 (*q*, α-CH_3–C(12)); 17.01 (*q*, CH_3–C(2)); 19.44 (*q*, CH_3–C(7)); 18.41 (*q*, CH_3–C(17)); 15.56 (*q*, CH_3–C(5)); 15.34 (*q*, CH_3–C(15)). FAB-MS ((2-nitro-phenyl)octylether): 1073 (32, M^+), 1047 (100, $M^+ - CN$), 1022 (23), 989 (40), 962 (25, $M^+ - 2\,CN - C_2H_5NO$), 945 (27), 928 (26), 902 (20), 888 (22), 874 (17), 855 (10). Anal. calc. for $C_{53}H_{72}CoH_7O_{13}$ (1074.1): C 59.21, H 6.75, N 9.12; found: C 59.33, H 6.70, N 8.77.

Hexamethyl (5R,6S)-Coα,Coβ-dicyano-7-de(carboxymethyl)-5,6,7^1,7^2-tetrahydro-5-hydroxy-7^2-oxopyrrolo[2,3-f]cobyrinate (**16**). Through a soln. of **13** (100 mg) in 25 ml of MeOH containing ascorbic acid (1 g), $KHCO_3$ (0.59 g), 40 ml of phosphate buffer (pH 7.2), and 5 ml of aq. EDTA (0.01M), a slow stream of O_2 was bubbled for 3 h at 70°. After cooling to r.t., the mixture was treated with 10 ml of 2% aq. KCN soln. and extracted with CH_2Cl_2 (2 × 30 ml). The combined org. layers were dried by filtration through cotton, the solvent was evaporated, and the residue was purified by prep. TLC using CH_2Cl_2/MeOH 96:4 which contained 0.1% of KCN. The product (35 mg, 34%) was removed from the silica gel with CH_2Cl_2/MeOH 90:10. Crystals suitable for X-ray analysis were obtained by keeping a soln. of **16** (30 mg) in dry MeOH (3 ml) in a Et_2O atmosphere for 10 days. After this time, the mother-liquor was clear, and 28 mg (24%) of dark-brown crystals (m.p. 249–251°, dec.) could be isolated. IR ($CHCl_3$): 3470*m*, 2990*m*, 2960*m*, 2125*w*, 1730*s*, 1700*s*, 1585*m*, 1535*m*, 1490*w*, 1440*m*, 1410*m*. UV/VIS: 485 (4.07), 462 (3.99), 335 (sh, 3.67), 319 (4.02), 303 (sh, 3.69). CD ($3.52 \cdot 10^{-5}$M): 489.5 (24878), 473 (19245), 458.5 (23939), 415 (0), 381 (–19714), 357 (0), 351 (5632), 331 (36143), 319 (52572), 285 (1408), 280.5 (2816), 276 (0), 263 (–21113), 254 (0), 240 (30041°). ^{1}H-NMR (360.13 MHz): 6.96 (*s*, NH); 5.06 (*s*, H–C(10)); 4.88 (*s*, HO–C(5)); 3.76, 3.75, 3.74, 3.70, 3.69, 3.64 (6*s*, 6 CH_3O); 2.15 (*s*, CH_3–C(15)); 1.86 (*s*, CH_3–C(5)); 1.47, 1.40, 1.32, 1.29, 1.23, 1.13 (6*s*, 6 CH_3). ^{13}C-NMR (90.56 MHz): 191.95 (*s*, C(4)); 175.89, 174.55, 174.35, 174.34, 174.30, 173.98, 172.93, 172.75, 171.79, 171.30 (10*s*, 6 $COOCH_3$, C(7^2), C(4), C(11), C(16)); 165.69 (*s*, C(14)); 99.25 (*s*, C(15)); 93.60 (*s*, C(6)); 85.50 (*d*, C(10)); 85.42 (*s*, C(1)); 79.46 (*s*, C(5)); 76.42 (*d*, C(19)); 59.75 (*d*, C(3)); 58.23 (*s*, C(17)); 56.45 (*d*, C(8)); 53.74 (*d*, C(13)); 53.12 (*s*, C(7)); 52.40, 52.35, 52.30, 52.00, 51.80, 51.62 (6*q*, 6 CH_3O); 46.35 (*s*, C(12)); 45.67 (*s*, C(2)); 44.74 (*t*, C(7^1)); 41.42 (*t*, C(2^1)); 39.93 (*d*, C(18)); 32.99, 32.74, 32.04, 31.84, 31.07, 29.85, 25.75, 24.25, 23.43 (9*t*, 9 CH_2); 31.18 (*q*, β-CH_3–C(12)); 25.74 (*q*, CH_3–C(5)); 21.90, 21.50 (2*q*, CH_3–C(1), CH_3–C(7)); 20.19 (*q*, α-CH_3–C(12)); 18.59 (*q*, CH_3–C(17)); 16.51 (*q*, CH_3–C(2)); 14.87 (*q*, CH_3–C(15)). FAB-MS (glycerol): 1063 (12, $M + 1)^+ - HCN$), 1037 (100, $M + 1)^+ - HCN - CN$), 1023 15), 1007 (8). FAB-MS (glycerol): 1089 (55, M^-), 1063 (73, $M^- - CN$), 1036 (100, $M^- - HCN - CN$), 1023 (868), 1004 (52). Anal. calc. for $C_{53}H_{72}CoN_7O_{14} \cdot H_2O$ (1108.1): C 57.43, H 6.73, N 8.85; found: C 57.39, H 6.73, N 8.82.

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