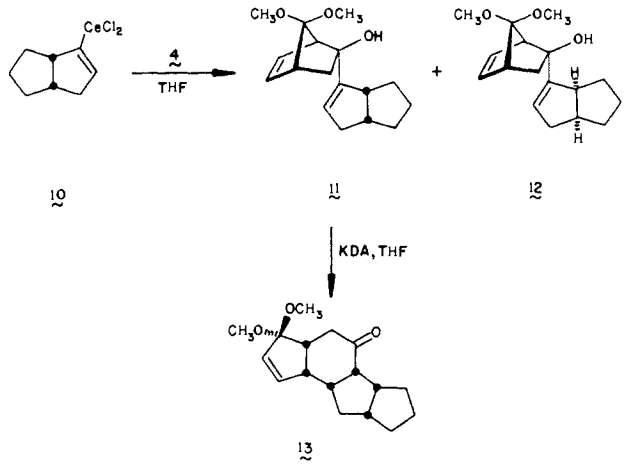
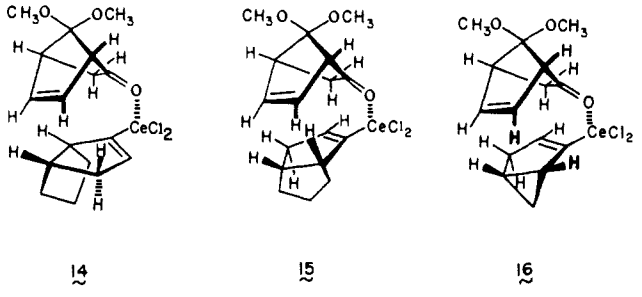
Scheme III



to be chromatographically separable. Furthermore, since 7,7-disubstituted 2-norbornenones are available in optically pure condition,[12] the preparation of **8**, **13**, and related ketones in high enantiomeric purity can potentially be easily realized.

The reasons for this synthetically useful[13] intermolecular recognition have not yet been fully elucidated. The methoxyl oxygens do not appear to play a significant coordinative role, since other functionality at C-7 leads to roughly comparable results.[14] Solvation factors are quite important,[15] and one-electron processes cannot be dismissed. If the Dunitz trajectory model[16] is assumed, with resultant stacking of the reactants as in **14**–**16**, then the



product ratios can be *satisfactorily* attributed to steric factors. On this basis, **15** (the combination leading to **12**) is disfavored because of nonbonded interactions not present in **14** (that culminating in production of **11**). Particularly intriguing is the triad of organocerium reagents derived from vinyl bromides A, B, and C, for which an appreciable falloff in diastereoselectivity is observed. Perhaps the increasing outward splaying brought on by small-ring annulation (see **16**) is responsible. Although this working hypothesis has predictive capability,[13] stereoelectronic effects are not accommodated and further exploration is highly desirable. Experiments aimed at elucidating some of these questions are in progress.[17]

(11) All of the vinyl bromides were prepared by application of the Shapiro reaction to the appropriate ketone tosylhydrazone precursor and condensation of the resulting vinyllithium reagent with cyanogen bromide. Compare: Barth, W.; Paquette, L. A. *J. Org. Chem.* **1985**, *50*, 2438.

(12) See, for example: (a) Grieco, P. A.; Takigawa, T.; Moore, D. R. *J. Am. Chem. Soc.* **1979**, *101*, 4380. (b) Grieco, P. A.; Owens, W.; Wang, C.-L. J.; Williams, E.; Schillinger, W. J.; Hirotsu, K.; Clardy, J. *J. Med. Chem.* **1980**, *23*, 1072.

(13) For an application to the synthesis of the tricyclic segment of the antibiotic ikarugamycin, see: Paquette, L. A.; Romine, J.; Lin, H.-S. *Tetrahedron Lett.*, in press.

(14) Romine, J., unpublished observations.

(15) Tetrahydrofuran is superior. Our findings on solvent effects will be discussed in the full paper.

(16) (a) Bürgi, H. B.; Dunitz, J. D.; Shefter, E. *J. Am. Chem. Soc.* **1973**, *95*, 5065. (b) Bürgi, H. B.; Dunitz, J. D. *Acc. Chem. Res.* **1983**, *16*, 153 and pertinent references cited therein.

(17) This research was supported by the National Institutes of Health (Grant GM-28468).

Structure of Factor S3, a Metabolite of *Propionibacterium shermanii* Derived from Uroporphyrinogen I

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During the last decade's research on the biosynthesis of vitamin B_{12}, three partially methylated intermediates have been isolated, viz. factors I (**2**), II (**3**), and III (**4**) corresponding to the introduction of one, two, and three methyl groups, respectively, from S-adenosylmethionine (SAM) into the reduced type III porphyrin template (**1**).[1] Recent studies on the sequence of methylation have revealed that eight methyl groups from SAM are inserted into uro'gen III (**1**) in the order C_2 (≡factor I) > C_7 (≡factor II) > C_{20} (≡factor III) > C_{17} > $C_{12\alpha}$ > C_1 followed by C_5/C_{15}[2,3] on the way to cobyrinic acid (**7**) (Scheme I). However, no intermediates beyond factor III have yet been discovered. In this paper we describe the isolation and structure of the zinc complex of a novel *tetramethylated* derivative of the porphyrinogen family whose UV/visible spectrum is closely related to that of the corresponding zinc corphinate (**8**).[4] The latter type of structure is unknown as a natural product but has been the subject of extensive synthetic studies by Eschenmoser.[4] It has been suggested[3,5] that both a corphin (such as **6**) and an isomeric pyrrocorphin (**5**)[2,3] may be intermediates in the biosynthesis of cobyrinic acid (**7**) from uro'gen III[3] (**1**).

Incubation of cobalt-deficient cell-free extracts of *Propionibacterium shermanii* with δ-aminolevulinic acid (ALA) in the presence of SAM followed by esterification ($MeOH/H_2SO_4$) and extensive preparative TLC afforded *four isomeric compounds* (factors S1–S4), each containing a UV/vis chromophore reminiscent of the synthetic model zinc corphinate (**8**).[4] The most abundant of these isomers, factor S3 (300 μg), was chosen for structural studies. FD- and FAB-MS revealed a molecular weight *m/z* 1102 ($C_{52}H_{67}O_{16}N_4ZnCl$) ($\xrightarrow{-Cl}$ 1067 $\xrightarrow{-Zn}$ 1004). Factor S3 is an octacarboxylic acid (FD-MS *m/z* octaethyl ester − octamethyl ester = 112 mass units) containing *four* methionine-derived methyl groups ($^{14}C/^{3}H$ ratios of factors derived from [$^{14}CH_3$]SAM + [2,3-$^{3}H_2$]ALA: F II:F III:F S3, **2** (0.29):**3** (0.44):**4** (0.58); FD-MS on a sample derived from CD_3-SAM showed addition

(1) Reviews: Scott, A. I. *Pure Appl. Chem.* **1981**, *53*, 1215; **1986**, *58*, 753; *Ann. N.Y. Acad. Sci.* **1986**, *471*, 174.

(2) Scott, A. I.; Mackenzie, N. E.; Santander, P. J.; Fagerness, P. E.; Müller, G.; Schneider, E.; Sedlmeier, R.; Wörner, G. *Bioorg. Chem.* **1984**, *12*, 356.

(3) Uzar, H.; Battersby, A. R. *J. Chem. Soc., Chem. Commun.* **1985**, 585. We have been unable to confirm the suggestion by these authors that methylation at C-5 and C-15 can be distinguished as $C_{15} > C_5$.

(4) Johnson, A. P.; Wehrli, P.; Fletcher, R.; Eschenmoser, A. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 623. Müller, P. M. *ETH Dissertation No. 5135*, 1973, Zurich. Eschenmoser, A. *Ann. N.Y. Acad. Sci.* **1986**, *471*, 108.

(5) Scott, A. I. *Proceedings of The Robert A. Welch Conferences on Chemical Research XXVIII. Chemistry in Texas: The 30th Year of The Welch Foundation*; Houston, TX, Nov, 1984. A corphin intermediate (cf. **9**) in B_{12} biosynthesis was invoked as early as 1973 (Scott, A. I.; Townsend, C. A.; Okada, K.; Kajiwara, M. *Trans. N.Y. Acad. Sci.* **1973**, *35*, 72).

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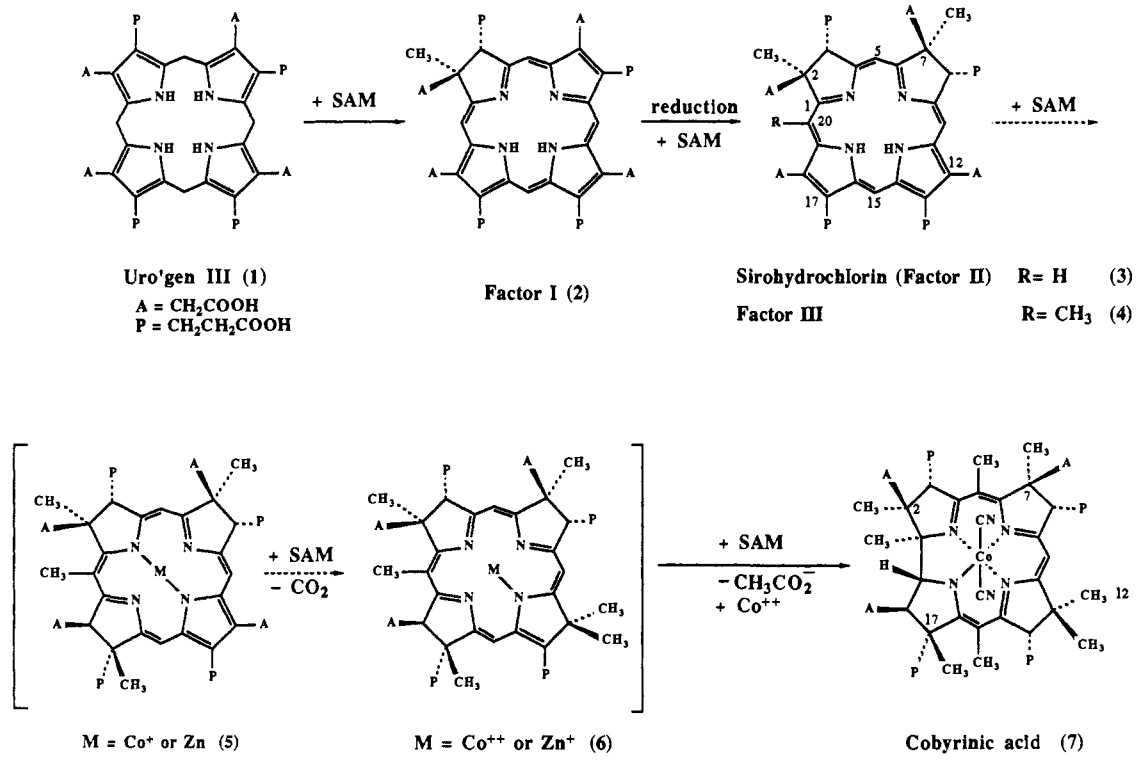
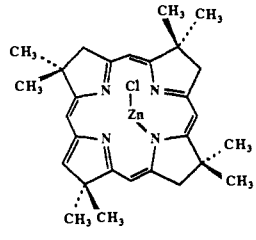
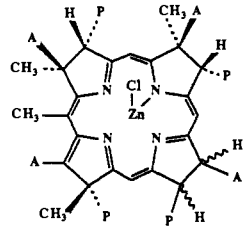
Scheme I



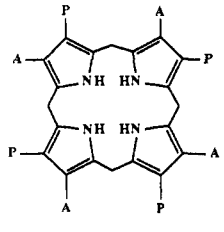
of 12 amu). Based on the above considerations, the most likely structure on biogenetic grounds is the zinc corphinate (**9**), i.e., the long-sought factor IV. However, the absence of strong fluorescence and the ratios of the UV/vis absorption maxima in the 300–560-nm region[6] suggest that factor S3 is, in fact, a close relative rather than a full member of the corphin family, a proposal which was confirmed by the following experiments.



(8)

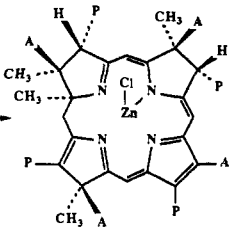


"Factor IV" (9)



Uro'gen I (11)

4 Methylations
SAM



Factor S3 (10)

Incubation of a cell suspension of *P. shermanii* in the presence of [4-^{13}C]ALA (90% ^{13}C) and [^{13}CH$_3$]-L-Met (90% ^{13}C)[7a] and isolation of the major pigments[7b] afforded, after esterification (MeOH–H_2SO_4) and multiple TLC, factor S3 (300 μg) enriched (~70%) with ^{13}C (FAB MS *m/z* 1114 ≡ M + 12). The proton-decoupled ^{13}C NMR spectrum showed temperature dependence and was recorded in $CDCl_3$ at −38 °C to remove the maximum number of tautomeric species. Under these conditions[8] signals for four methyl groups (δ 16–24) and for eight enriched carbons derived from C-4 of ALA were observed. Of particular note are the signal at 79 ppm (>C–N) and two resonances in the sp^3 region (45–50 ppm) shown to be methines (>C–H) by off-resonance decoupling. The remaining five ALA-derived resonances (▲) are assigned to sp^2 hybridized carbons (130–180 ppm) (see Figure 1a). An INADEQUATE[9] experiment (Figure 1b) reveals that only one SAM-derived methyl group (*; 22 ppm) is coupled (J = 54 Hz) to the (▲) C-1 quaternary carbon (>C–N) at 79 ppm. Further structural assignments were made by preparing two additional versions of the ^{13}C-enriched zinc complex. The NMR spectrum of factor S3 octamethyl ester (70 μg) derived from [^{13}C-5]ALA and [^{13}CH$_3$]SAM surprisingly exhibited a ^{13}C–^{13}C coupling pattern expected for a type I rather than a type III derivative of porphyrinogen, since the eight [5-^{13}C]ALA derived resonances (■) (C) were observed in the sp^2/sp^3 region as coupled *pairs* (Figure 1a). No contiguous trio, the hallmark of type III porphyrinogens[10] and of isobacteriochlorins,[1] was present. The molecule therefore belongs to the *symmetrical* type I series. This result, taken together with the absence of *any* H_3C–C coupling and a [^{13}C-5]ALA-derived resonance at 35 ppm (C-20; (■) CH_2) coupled (J = 37 Hz) to >C=N (■) C-19; 147 ppm), leads to structure **10** as one of the possible representations for factor S3, i.e., a tetramethylated type I derived *isomer* of a zinc corphinato chloride[11] derived from ALA via **11**. The stereochemistry shown in **10** is based on analogy with the structures of factors I–III

(6) Factor S3 shows λ_{max}(EtOH) 268 (relative intensity = 0.30), *302* (0.67), 359 (0.92, s), *369* (1.00), 415 (0.13, s), *537* (0.38). Tetramethyl zinc corphinate[4] (**8**) has λ_{max}(EtOH) 263 (ε = 11 500), 293.5 (38 000, s), *302* (48 100), 343.5 (40 000, s), *358* (49 400), 384 (6300, s), 411.5 (4450), *531* (9775) nm.

(7) (a) Typically, wet cells, 100 g, of *P. shermanii* suspended in 300 mL of degassed phosphate buffer (pH 7.7) were incubated under strictly anaerobic conditions with ALA·HCl (50 mg, 300 mol), L-Met (50 mg, 337 μmol) + $ZnSO_4$ (16 mg, 100 μmol) during 48 h, with regular pH adjustment. Tetrapyrrolic compounds were isolated[7b] under argon. Multiple TLC on different adsorbents (silica gel, alumina) yielded 27 μg of factor S3. (b) Bergmann, K.-H.; Deeg, R.; Gneuss, K.-D.; Kriemler, H.-P.; Müller, G. *Hoppe-Seyler's Z. Physiol. Chem.* **1977**, *358*, 1315.

(8) Professor A. Eschenmoser, private communication.

(9) Bax, A.; Freeman, R.; Kempsell, S. P. *J. Am. Chem. Soc.* **1980**, *102*, 4849.

(10) Scott, A. I. *Acc. Chem. Res.* **1978**, *11*, 29.

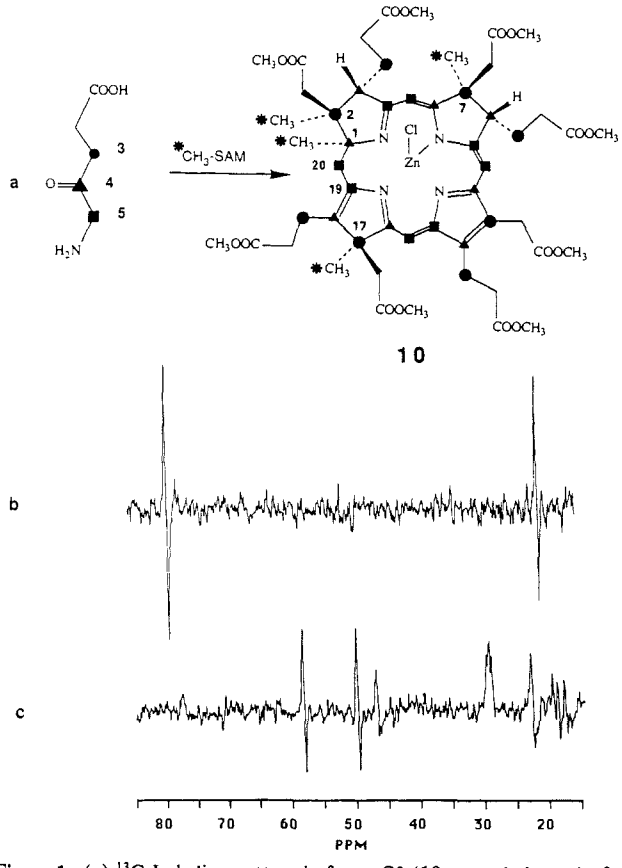


Figure 1. (a) ^{13}C-Labeling pattern in factor S3 (**10** as methyl ester) after incorporation of (*) $^{13}CH_3$ L-Met (or SAM) and (●) [3-^{13}C]-, (▲) [4-^{13}C]-, and (■) [5-^{13}C]ALA in three separate experiments. The proton-decoupled ^{13}C-NMR spectra (not shown) of each specimen revealed *four* $^{13}CH_3$- and *eight* ALA-derived signals. For details see text. (b) INADEQUATE ^{13}C spectrum of factor S3 (300 μg) derived from [4-^{13}C]ALA and [$^{13}CH_3$]-L-Met (75.4 MHz, $CDCl_3$, −38 °C) showing coupling (J = 54 Hz) between (*) CH_3 (21 ppm) and (▲) C-1 (79 ppm). (c) INADEQUATE ^{13}C spectrum of factor S3 (150 μg) derived from [3-^{13}C]ALA and [$^{13}CH_3$]SAM (75.4 MHz, $(CD_3)_2CO$, −40 °C) showing (*) CH_3 − (●) C couplings (J = 35 Hz) for the three remaining methyl groups ((*) CH_3; δ 16–24) attached to enriched quaternary carbons at C-2, C-7, and C-17 ((●) C; δ 45–60). The signal at 29 ppm is due to acetone.

and remains to be proved. Rigorous confirmation that *three* of the four "extra" SAM-derived methyl groups are attached to quaternary carbons bearing *acetate* side chains was forthcoming when a third isotopomer of factor S3 (150 μg) was isolated from an incubation with [^{13}C-3]ALA (99% $^{13}C^{12a}$) and [$^{13}CH_3$]SAM. The ^{13}C INADEQUATE spectrum of the octamethyl ester of this specimen (Figure 1c) reveals three one-bond ^{13}C–^{13}C couplings (J = 35 Hz) corresponding to methylation at the *acetate* termini of three of the β-positions of the original pyrrolic rings of uro'gen I (**11**). The fourth methyl group (at C-1) and the five remaining enrichments (●) (C) from [^{13}C-3]ALA are "silent" in the INADEQUATE spectrum as required by structure **10** (Figure 1a). Incorporation experiments with ^{3}H- and ^{14}C-labeled factor S3 as a potential precursor of vitamin B_{12} were negative, in accord with the proposed structure.

Although not on the pathway to vitamin B_{12}, factor S3 represents the first example of biological methylation of uroporphyrinogen I (**11**)[13] and is highly suggestive of the mode of incorporation of methyl groups into the more familiar type III nucleus, including both β- (C-2, C-7, C-17) and α-alkylation (C-1). The fact that its ^{13}C NMR spectra display temperature-dependent tautomeric flux places factor S3 in the same category as Eschenmoser's zinc corphinate (**8**) whose ^{13}C spectrum is completely resolved only at −35 °C,[8] and this phenomenon forms an important part of the structure proof for **10**. At present the biological roles of uroporphyrinogen I and factors S1–S4 are unknown, but the methylations responsible for the production of factor S3 in cell-free extracts and in whole cells of *P. shermanii*[14] must surely be related to the B_{12} pathway, suggesting that the elusive factor IV may indeed have structure **9**, i.e., a 2,7,17,20-tetramethyl zinc corphinate.

Acknowledgment. We thank the National Institutes of Health (AM32034), the Robert A. Welch Foundation, Deutsche Forschungsgemeinschaft and N.A.T.O. for generous support of this work and Professor Albert Eschenmoser for helpful discussions.

(11) Structure **10** represents one of four regioisomers corresponding to methylation at each α-pyrrolic position. For the sake of simplicity we have illustrated only one of the four possible isomers, each of which is susceptible to tautomeric flux, exchange of Cl at the axial positions, *and* epimerization at the two asymmetric centers bearing propionate side chains.

(12) (a) [3-^{13}C]ALA was obtained by a modification of Eschenmoser's procedure.[12b] (b) Pfaltz, A.; Juan, B.; Fässler, A.; Eschenmoser, A.; Jaenchen, R.; Gilles, H. H.; Dickert, G.; Thauer, R. K. *Helv. Chim. Acta* **1982**, *65*, 828.

(13) Methylation of uro'gen I by a partially purified methylase from *P. shermanii* has been described (Müller, G. In *Vitamin B_{12}*; Zagalak, B., Friedrich, W., Eds.; de Gruyter: New York, 1979; p 279), but the product was not characterized.

(14) Müller, G., unpublished results.