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Supplementary Material Available: IR, ^{1}H NMR, ^{13}C NMR, mass spectra, and analytical data for **1**, **3–16**, and the hydrolysis product from **16** (5 pages). Ordering information is given on any current masthead page.

Structure of "Dioneheme". Total Synthesis of the Green Heme Prosthetic Group in Cytochrome cd_1 Dissimilatory Nitrite Reductase

Weishih Wu and C. K. Chang*

Department of Chemistry, Michigan State University
East Lansing, Michigan 48824

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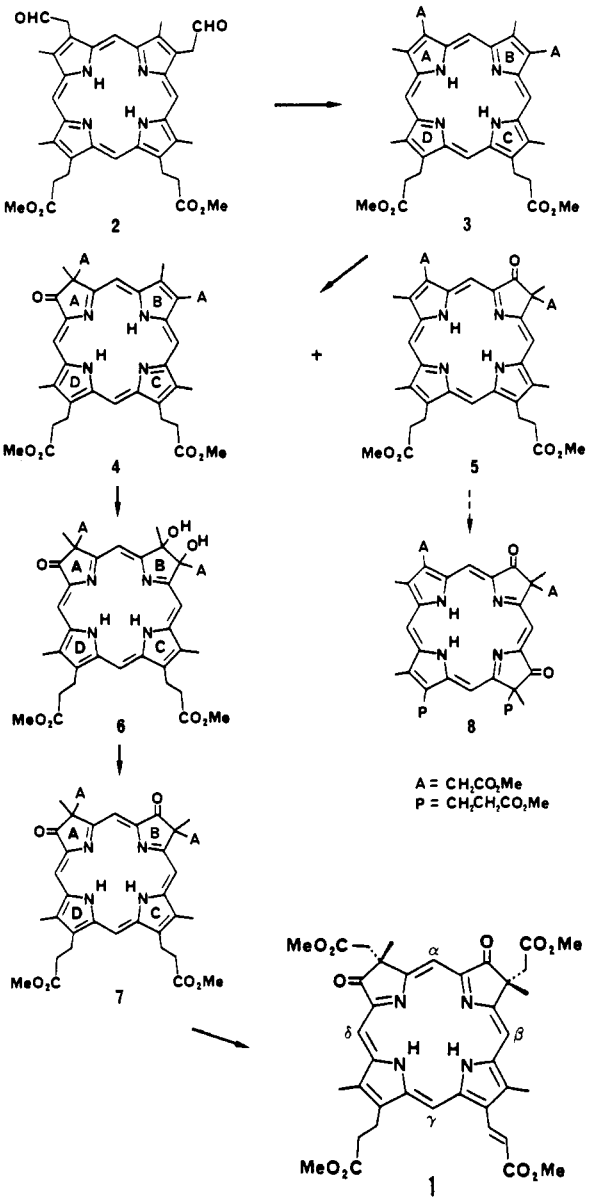
Bacterial denitrification which may have generated all of the nitrogen in the earth's atmosphere now maintains N_2 levels by continually reducing nitrates from soil and water.[1,2] A key redox enzyme occurring in many denitrifying bacteria is called cytochrome cd_1 which mediates the 4e reduction of nitrite to N_2O. In the absence of nitrite, this enzyme also functions as an oxidase reducing O_2 to H_2O.[3] A unique feature of this cytochrome is that it contains, in 1:1 ratio to the protein-linked heme *c*, a green, substrate-binding heme prosthetic group known as heme d_1.[4] Although the presence of this unusual heme has been demonstrated since 1961,[5] its chemical identity remained obscure. Timkovich and co-workers in 1984 presented detailed ^{1}H NMR, UV–vis, and mass spectra of the extracted and demetalated d_1 heme moiety.[6] They concluded that it has a chlorin core structure. We, however, proposed that a porphinedione (dioxoisobacteriochlorin) structure would fit the spectral data better and therefore d_1 is not a chlorin.[7] Strong support in favor of our unconventional structure (**1**) has been accumulating during the past year, drawing from various spectral correspondence between the natural pigment and synthetic analogues possessing the proposed dione nucleus.[8,9] While these spectral evidence have effectively argued that **1** must be correct, the ultimate proof of structure could only come from total synthesis or X-ray crystallography, as common in the natural products chemistry. We have now achieved this objective and report here the synthesis of the metal-free "dioneheme"[10] tetramethyl ester.

The pinacol–pinacolone-type rearrangement of *vic*-dihydroxychlorin is a proven method for introducing geminal alkyl as well as keto groups at the porphyrin periphery.[11,12] The knowledge

Scheme I



of migratory aptitudes of different substituents associated with such rearrangement[13] also helped to formulate the synthetic plan. It was decided that double migration of the northern methyl groups in porphyrin **3** would lead to the target dione structure. Thus the dialdehyde **2**,[14] obtained by $Tl(NO_3)_3$/MeOH oxidation of protoporphyrin, was oxidized by Jones reagent to provide the starting porphyrin **3** in 92% yield. **3** was treated with osmium tetroxide in CH_2Cl_2/pyridine and quenched by H_2S after 24 h to obtain dihydroxylation occurring at all four pyrrole rings. The two northern diols (ring A and B, 20%) were separated from the southern diols (ring C and D, 35%) on silica gel and were subjected to the first rearrangement in 1:1 FSO_3H/H_2SO_4;[15] the resultant monoketones **4** and **5** were separated by chromatography. The

(1) Payne, W. J. *Denitrification*; Wiley: New York, 1981.
(2) Delwiche, C. C. *Denitrification, Nitrification, and Atmospheric Nitrous Oxide*; Wiley: New York, 1981.
(3) Greenwood, C. In *Metalloproteins*; Harrison, P. M., Ed.; Verlag Chemie: Weinheim, FRG, 1985: Part I, pp 43–77.
(4) (a) Kuronen, T.; Ellfolk, N. *Biochim. Biophys. Acta* **1972**, *275*, 308. (b) Gudat, J. C.; Singh, J. S.; Wharton, D. C. *Biochim. Biophys. Acta* **1973**, *292*, 376.
(5) (a) Yamanaka, T.; Okunuki, K. *Biochim. Biophys. Acta* **1961**, *67*, 379. (b) Horio, T.; Higashi, T.; Yamanaka, T.; Matsubara, H.; Okunuki, K. *J. Biol. Chem.* **1961**, *236*, 944.
(6) (a) Timkovich, R.; Cork, M. S.; Taylor, P. V. *J. Biol. Chem.* **1984**, *259*, 1577. (b) Timkovich, R.; Cork, M. S.; Taylor, P. V. *J. Biol. Chem.* **1984**, *259*, 15089.
(7) Chang, C. K. *J. Biol. Chem.* **1985**, *260*, 9520.
(8) Chang, C. K.; Wu, W. *J. Biol. Chem.* **1986**, *261*, 8593.
(9) (a) Chang, C. K.; Timkovich, R.; Wu, W. *Biochemistry* **1986**, *25*, 8447. (b) Andersson, L. A.; Loehr, T. M.; Wu, W.; Chang, C. K.; Timkovich, R., submitted for publication in *J. Am. Chem. Soc.*
(10) Suggested trivial name for d_1 heme, see ref 8.
(11) (a) Inhoffen, H. H.; Nolte, W. *Justus Liebigs Ann. Chem.* **1969**, *725*, 167. (b) Chang, C. K. *Biochemistry* **1980**, *19*, 1971. (c) Chang, C. K.; Wu, W. *J. Org. Chem.* **1986**, *51*, 2134.
(12) Chang, C. K.; Sotiriou, C.; Wu, W. *J. Chem. Soc., Chem. Commun.* **1986**, 1213.
(13) Chang, C. K.; Sotiriou, C. *J. Heterocycl. Chem.* **1985**, *22*, 1739.
(14) Kenner, G. W.; McCombie, S. W.; Smith, K. M. *Justus Liebigs Ann. Chem.* **1973**, 1329.
(15) The pinacolic rearrangements involving acetate substituents were plagued with poor yield and byproduct. In the first rearrangement, the conditions were optimized by using superacid FSO_3H/H_2SO_4/fuming H_2SO_4 (10:10:1) which gave about 55% of the monoketone **4** or **5**. If concentrated H_2SO_4 was used alone, yield was only 15% with the major product being the γ-lactone which resists rearrangement or hydrolysis. Using Nafion or Magic acid, only decomposition was observed. In the rearrangement of **6**, however, the presence of FSO_3H catalyzed the reduction of **6** to **4**.

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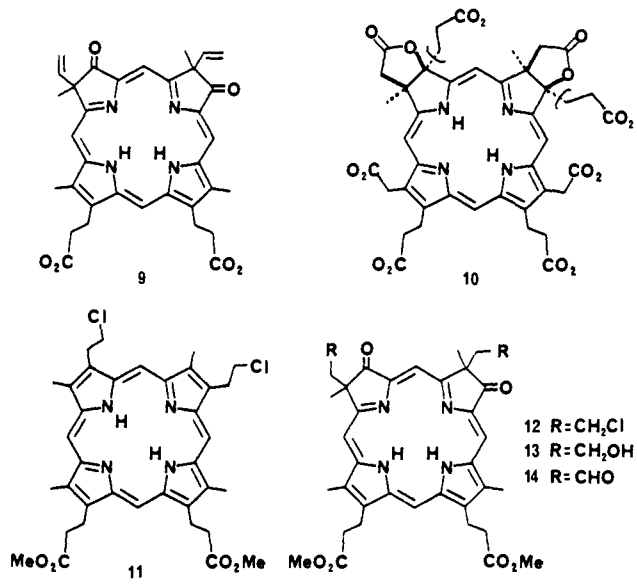
southern diols could be reduced by $HOAc/HI/H_3PO_2$ and converted[16] quantitatively back to **3**, together with recovered **3** (40%), for recycling. The yield of **4** was about 6% from **3** each time.

Porphyrinone **4** was metalated with zinc(II) acetate in $CHCl_3$/MeOH and the zinc complex[12] was oxidized with OsO_4. Following zinc removal by HCl washing, the desired diol **6** was isolated by chromatography (18% yield), with byproducts being the ring C (15%) and ring D diols (12%). We reported earlier[12] that analogous zinc(II) porphyrinones substituted with electron-rich alkyl groups exhibit a differentiation during osmate formation favoring ring B but against ring D attack; in addition, the ring C diol would not form at all. In the present case, the electronegative acetate side chain apparently has rendered the ring B attack less favorable, thereby overiding the empirical rule observed with other compounds. The presence of the acetate substituent further hindered the second pinacolic rearrangement at ring B so that the yield of **7** was a disappointing 12% under the best conditions tested.[15] In contrast, the same reaction sequence applied to the zinc complex of **5** produced overall nearly 10% yield of **8**.

The diastereomeric mixture of **7** was separated on silica gel TLC plates. The isolated cis and trans isomers each were converted by OsO_4 into a ring C diol which was then heated in benzene in the presence of HCl[8,17] to obtain the acrylate **1**. TLC and HPLC confirmed that the slower eluting, presumably cis isomer is indeed identical with the natural d_1 tetramethyl ester. The ^1H NMR of the two isomers showed recognizable differences and only the spectrum of the cis compound is in every respect identical with that of the natural pigment.[18] Absorption and mass spectra further confirmed fully what we considered to be the correct structure of d_1. Future work on resolving the racemic mixture should aid in elucidating the natural molecule's absolute configuration.

The unprecedented structure of dioneheme poses many questions. Is there a functional role of the oxo groups at the isobacteriochlorin ring? How is this structure produced biosynthetically? To the former question we have observed that the relatively positive redox potentials[19] of porphyrinone and porphyrindione distinguish them from chlorin-based heme d[20] or isobacteriochlorin-based siroheme[21] and render these keto macrocycles perhaps more like porphyrin "quinones", which may be of consequence in nitrite binding and reduction.[8] To speculate on the second question, we previously suggested pinacolic rearrangements may have been involved in the biosynthesis.[7] However, in view of the difficulties experienced in the rearrangement of porphyrin acetates,[15] a porphyrin precursor such as **3** seems less plausible. An alternative precursor could be protoporphyrin or its derivative from which a dione structure (**9**) may undergo side-chain oxidation to furnish the acetate substituents. This approach has actually been tried in one of our unsuccessful attempts to synthesize **1**. The vinyl porphyrin in the masked form of chloroethyl porphyrin **11** was processed via exactly the same steps as described above, only with much better yields, to give **12**. Substitution of the Cl by NaOH and oxidation of the diol **13** by the Swern[22] method afforded the dialdehyde **14**. Unfortunately the macrocycle did not survive the oxidants necessary for converting -CHO into $-CO_2$. This route would have been a success if milder conditions could be found. Biosynthesis without invoking



pinacolic rearrangement is also possible. Indeed, the stereochemistry and side-chain substitution patterns suggest that sirohydrochlorin or corriphyrin-4 (**10**)[23] could be the progenitor if suitable avenues exist for cleaving the two northern propionate side chains. While we are presently short of answers to these questions, it can be predicted that the striking structural features uncovered here will not fail to draw attention and to stimulate further research on this green heme.

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(16) (a) Fischer, H.; Eckoldt, H. *Justus Libigs Ann. Chem.* **1940**, *544*, 159. (b) Chang, C. K.; Sotiriou, C. *J. Org. Chem.* **1985**, *50*, 4989.
(17) Chang, C. K.; Sotiriou, C. *J. Org. Chem.* **1987**, *52*, 926.
(18) Measured at 250 MHz in $CDCl_3$ with a standard concentration of 3 mM. Prominent differences are in the α,β-meso protons (cis 8.418 (trans 8.363), 8.266 ppm (8.204)), ring B methyl (1.776 ppm (1.735)), and the acetate methyls (3.127 (3.217), 3.176 ppm (3.209)). All chemical shifts of the synthetic cis compound fall within ±0.007 ppm of the published data of Timkovich's d_1.[6,9a] Assignments were based on NOE connectivities.
(19) Chang, C. K.; Barkigia, K. M.; Hanson, L. K.; Fajer, J. *J. Am. Chem. Soc.* **1986**, *108*, 1352.
(20) Vavra, M. R.; Timkovich, R.; Yap, F.; Gennis, R. B. *Arch. Biochem. Biophys.* **1986**, *250*, 461.
(21) Chang, C. K. In *The Biological Chemistry of Iron, NATO ASI Series* Dunford, H., Ed.: D. Reidel: Dordrecht, 1982; pp 313-334.
(22) Mancuso, A. J.; Brownfain, D. S.; Swern, D. *J. Org. Chem.* **1979**, *23*, 4148.
(23) (a) Battersby, A. R.; McDonald, E. *Bioorg. Chem.* **1978**, *7*, 161. (b) Scott, A. I.; Irwin, A. J.; Siegel, L. M.; Shoolery, J. N. *J. Am. Chem. Soc.* **1978**, *100*, 7987.

Stochastic Exploration of Molecular Mechanics Energy Surfaces. Hunting for the Global Minimum

Martin Saunders

Department of Chemistry, Yale University
New Haven, Connecticut 06520

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The use of molecular mechanics has increased in recent years as organic chemists continue to focus on important questions of stereochemistry.[1] In dealing with flexible molecules of any size or complexity, a major difficulty arises which may be described as the *local* vs. *global* minimum problem. If one enters a trial structure using any standard molecular mechanics program, optimization occurs to refine the starting structure toward one of lower steric energy, converging when a minimum energy structure is obtained. But how can one know the structure obtained is the *best* structure using that force field?

In small molecules, there are few conformations, and we can predict which are most stable. However, adding substituents or additional atoms to rings or chains rapidly takes us to situations where in the words of Allinger,[1] "The number of conformations becomes so large that a complete analysis becomes very laborious. The results depend not so much on the force-field as on the intuition of the person doing the calculation and which starting geometries were used for the energy minimizations".

(1) Burkert, O.; Allinger, N. L. *Molecular Mechanics*; ACS Monograph 177; American Chemical Society: Washington, DC, 1982.

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