Table I. Fractional ^{1}H NMR Areas and Equilibrium Constants (35 °C) of *anti*-7-Chlorobicyclo[4.3.2]undecatetraene-d_1 (**1**-d_1)

structural assignment:		vinyl	α	bridgehead			
δ_H, ppm[a]		6.10–5.45	4.80–4.70	3.65–3.40			
areas calcd for random distribution:		0.727	0.091	0.182			
precursor	reaction conditions	fractional areas obsd[b]			Isolated yield[c]	$K_{\alpha B}$[b]	$K_{\alpha V}$[b]
1. *syn*-**3**-d_0	$SOCl_2$, pyr/ether	0.725 (3)	0.092 (2)	0.184 (1)			
2. *anti*-**2**-d_0	$SOCl_2$, pyr/ether	0.730 (3)	0.091 (1)	0.179 (2)			
3. *syn*-**3**-*8*-d_1	$SOCl_2$, pyr/ether	0.749 (6)	0.083 (4)	0.168 (4)	98%	1.1 (3)	2.7 (6)
4. *syn*-**3**-*8*-d_1	MesCl Et_4NCl, Et_3N	0.748 (3)	0.079 (2)	0.173 (2)	97%	1.6 (2)	3.3 (3)
5. *syn*-**3**-*7*-d_1	$SOCl_2$, pyr/ether	0.763 (5)	0.073 (5)	0.167 (2)	100%	1.4 (2)	6 (2)
6. *anti*-**2**-*8*-d_1	$SOCl_2$, pyr/ether	0.746 (6)	0.085 (2)	0.169 (6)	98%	1.00 (6)	2.2 (2)
7. *anti*-**2**-*8*-d_1	MesCl, Et_4NCl, Et_3N	0.743 (4)	0.086 (2)	0.171 (3)	94%	1.0 (3)	2.0 (4)
8. *anti*-**2**-*7*-d_1	$SOCl_2$/pentane	0.754 (3)	0.073 (5)	0.172 (5)	94%	1.7 (3)	3.8 (6)
9. *anti*-**2**-*7*-d_1	$SOCl_2$/pyr, ether	0.757 (7)	0.076 (2)	0.167 (2)	100%	1.6 (1)	4.3 (5)
10. *anti*-**2**-*7*-d_1	$(ClCO)_2$/PhH(vac)[d]	0.747 (4)	0.080 (3)	0.172 (1)	*e*	1.8 (3)	3.6 (7)
11. *anti*-**2**-*7*-d_1	$(ClCO)_2$/PhH	0.750 (1)	0.076 (2)	0.174 (1)	*e*	1.5 (2)	3.0 (7)
12. *anti*-**2**-*7*-d_1	$(ClCO)_2$/PhH	0.744 (3)	0.079 (3)	0.176 (2)	*f*	1.83 (9)	2.9 (3)
13. *anti*-**1** (from 12)	$SnCl_4$, CH_2Cl_2	0.743 (3)	0.077 (3)	0.181 (3)	*f*	2.2 (1)	3.1 (3)
	weighted mean[g]	0.749 (1)	0.079 (1)	0.172 (1)		1.48 (4)	3.0 (1)

[a] Continuous wave spectra in $CDCl_3$ at 90 MHz except where otherwise specified. [b] Mean and standard deviations in the last digit as obtained from 3–5 scans. Except where otherwise specified, all samples were purified via HPLC. [c] Prior to HPLC purification. [d] Vacuum line techniques were used. Inert atmosphere syringe techniques were used elsewhere. [e] Analysis without prior HPLC purification. [f] FT spectra at 80 MHz. [g] $\bar{x} = \Sigma(x_i/s_i)/\Sigma(1/s_i)$ and $s = 1/(\Sigma 1/s_i^2)^{1/2}$.

structural isomerization of **1**-d_1 to **4**-d_1 was catalyzed by stannic chloride, and the process was halted after 60% completion. Recovered unreacted **1**-d_1 should then have had ample opportunity for reversible chloride dissociation to be complete. Because the resulting isotopic distribution remained unchanged, it seems reasonable to identify its persistence with chemical equilibrium.

Equations 1 and 2 were then used to define and evaluate the two equilibrium constants.[13] Each n_i represents the mole fraction

$$K_{\alpha B} \equiv \frac{2n_\alpha}{n_B} = \frac{a_V - 9a_\alpha + a_B}{a_V + a_\alpha - 4a_B} \quad (1)$$

$$K_{\alpha V} \equiv \frac{8n_\alpha}{n_V} = \frac{4a_V - 36a_\alpha + 4a_B}{-a_V + 4a_\alpha + 4a_B} \quad (2)$$

of one isotopic isomer with its deuterium atom at site i; the a_i are the corresponding observed areas. Because the algebraic form of these equations attenuates experimental precision, nonrandom error propagation was avoided by evaluating $K_{\alpha B}$ and $K_{\alpha V}$ separately for each NMR scan. To the extent that precursors were incompletely deuterated, 1.48 and 3.0 should be regarded as *lower limits* of the true equilibrium constants. One recalls too that $K_{\alpha V}$ is an average over 8 nonequivalent sites; some of them might well require larger equilibrium isotope effects than the rest.[14]

Why are these isotope effects so large? Molecular models reveal no apparent steric constraints about the α hydrogen, but constraints might well be present nonetheless. Nucleophiles attack the corresponding ketone almost exclusively from the anti direction.[15] Perhaps the preference for bridgehead to vinyl deuteration ($K_{BV} = K_{\alpha V}/K_{\alpha B} = 2.0$) is greater than that in the cyanobicyclo[4.2.0]octatrienes, because there the CH bond is also a less constrained cyclobutyl CH bond.[16]

A more interesting possibility is that these isotope effects only appear to be large for want of adequate comparison. If so, they might profitably encourage discovery of still larger effects in molecules that provide correspondingly greater contrasts in local force fields. Empirical calibration of secondary deuterium *kinetic* isotope effects could then rest more securely upon a wider range of structurally well-defined equilibrium isotope effect data.

Acknowledgment. We are grateful to E. S. Lewis and V. J. Shiner, Jr., for critical comments and to the National Science Foundation for its contributions to research (CHE77-26482) and to the purchase of an NMR spectrometer (CHE76-05884). Spectra were also obtained at the Northeast Regional NSF-NMR Facility (270 MHz) and, through the courtesy of E. R. Stimson, at Carnegie-Mellon University (600 MHz).

Supplementary Material Available: A brief derivation of eq 1 and 2 (1 page). Ordering information is given on any current masthead page.

(13) See supplementary material. A more general derivation will be presented elsewhere. The critical reader can verify eq 1 and 2 at the extremes; e.g., $K_{\alpha B} = K_{\alpha V} = 1$ when $a_V = 8$, $a_\alpha = 1$, and $a_B = 2$.

(14) 270-MHz NMR spectra resolved the two bridgehead protons and showed them to be of equal area. The vinyl protons remained incompletely resolved, even at 600 MHz.

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Formation of *N*-Phenylheme in the Hemolytic Reaction of Phenylhydrazine with Hemoglobin

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The formation of a green pigment in erythrocytes of animals treated with phenylhydrazine was described by Hoppe-Seyler in 1885.[1] Pigment formation has subsequently been found to be intimately associated with the precipitation of hemoglobin in the form of Heinz bodies and with the ensuing hemolytic anemia.[2] Despite a century of continuous scrutiny, however, the nature of the green chromophore and the mechanism by which it is formed remain unknown. The green substance was actually isolated in

(1) Hoppe-Seyler, G. *Z. Physiol. Chem.* **1885**, *9*, 34–39.

(2) (a) Beutler, E. *Pharmacol. Rev.* **1969**, *21*, 73–103. (b) Webster, S. H. *Blood* **1949**, *4*, 479–497.

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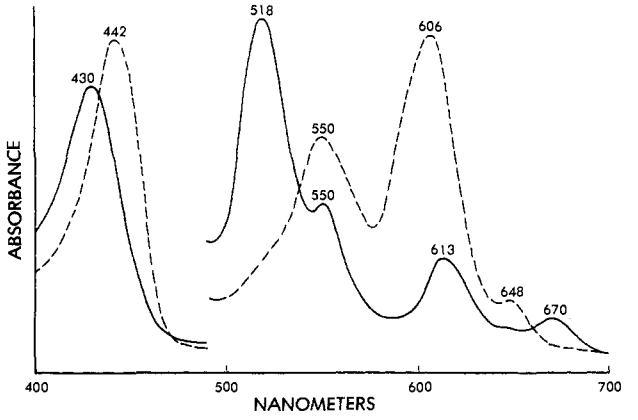

Figure 1. Electronic absorption spectrum in CH_2Cl_2 of the purified green pigment from phenylhydrazine-treated hemoglobin (a) before (solid line) and (b) after (dashed line) complexation with divalent zinc. The Soret bands are recorded at a tenfold higher attenuation.

Reaction of phenylhydrazine·HCl (500 mg) with human hemoglobin (Sigma Chemical Co., 2.0 g) in 100 mL of phosphate buffer (0.1 M, pH 7.4) for 2 h, followed by workup in acidic methanol,[5] yielded a crude green-brown (red fluorescing) pigment.[6] No pigment was obtained in the absence of oxygen. The pigment was treated with zinc acetate to form the zinc complex[5] before purification by sequential thin-layer and high-pressure liquid chromatography (HPLC).[7] The latter procedure resolves the pigment into two fractions, each of which contains two still unresolved regioisomers of a single structure (four isomers altogether) (vide infra).

The absorption spectra of the purified but unresolved isomer mixture (Figure 1) are characteristic of a porphyrin structure. Essentially the same spectra are obtained if the pigment is first resolved into two fractions by HPLC. The field desorption mass spectrum of each of the two metal-free fractions exhibits peaks at m/e 666 (M^+) and 667 ($M^+ + 1$). These molecular ion data are consistent with a structure in which a phenyl moiety (M_r 77) is covalently bound to dimethyl esterified protoporphyrin IX (M_r 590). The NMR spectrum of the zinc-complexed, unresolved, isomer mixture (Figure 2) exhibits three signals in addition to

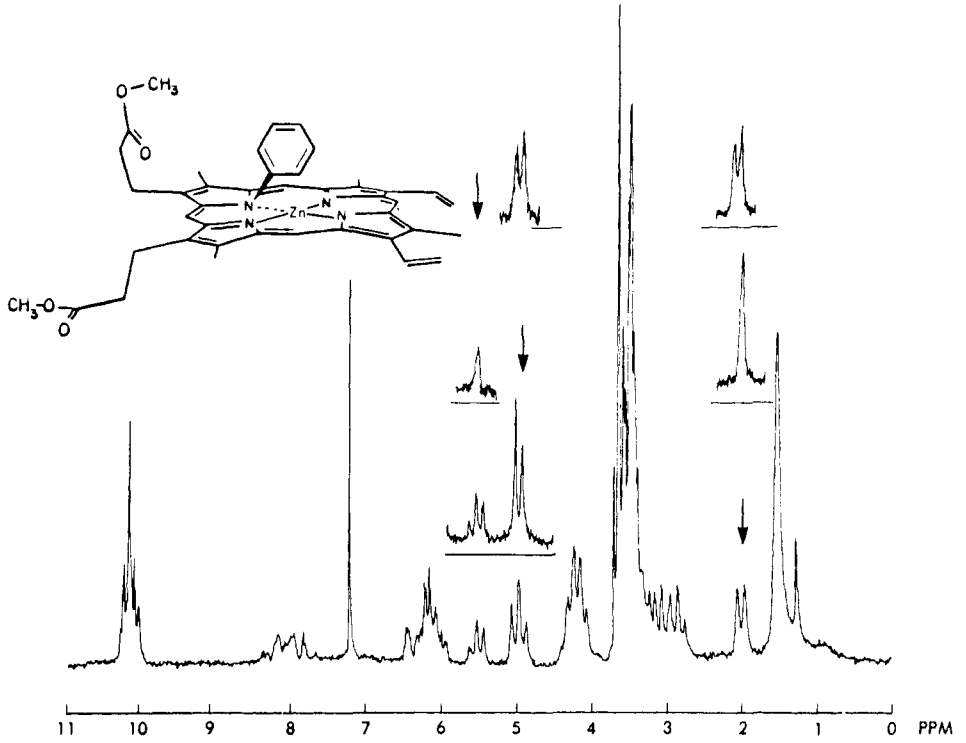

Figure 2. NMR spectrum of the mixture of zinc-complexed *N*-phenylprotoporphyrin IX (dimethyl ester) isomers isolated from phenylhydrazine-treated hemoglobin. The singlet at 7.27 ppm is due to $CHCl_3$, and the peaks at 1.6 and 1.3 ppm are due to water and impurities. The change in the spectrum on irradiation of the indicated signal (arrow) is shown on the same inset base line. The structure of one of the four isomers present in the sample is shown.

1954 but was not purified or characterized.[3] More recent studies have concentrated on the mechanism which leads to hemoglobin precipitation and have generally dealt with the chromophore within the protein matrix rather than as a distinct molecular entity.[4] We report here identification of the isolated green chromophore as *N*-phenylprotoporphyrin IX and chemical synthesis of this first *N*-arylporphyrin.

those assignable[8] to the protons of the original protoporphyrin IX skeleton of heme: 5.50 (t, 1 H), 4.95 (t, 2 H), and 2.02 ppm (d,

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(4) For example: (a) Peisach, J.; Blumberg, W. E.; Rachmilewitz, E. A. *Biochim. Biophys. Acta* **1975**, *393*, 404–418. (b) Itano, H. A.; Hirota, K.; Vedvick, T. S. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 2556–2560. (c) Goldberg, B.; Stern, A. *Arch. Biochem. Biophys.* **1977**, *178*, 218–225. (d) French, J. K.; Winterbourn, C. C.; Carrell, R. W. *Biochem. J.* **1978**, *173*, 19–26.

(5) The metal ligand is lost, and the carboxyl groups of the porphyrin are methylated, in acidic methanol. Addition of zinc acetate leads to formation of the zinc complex, from which the free base can again be liberated by acidic methanol treatment. For HPLC and NMR spectroscopy, the acetate counterion was replaced by chloride.[14c] The details of these procedures have been reported.[14]
(6) The same green pigment is also obtained from pure oxyhemoglobin, oxymyoglobin, methemoglobin, or metmyoglobin.
(7) TLC: silica gel G, 5:1 $CHCl_3$–acetone. HPLC: 9.4- x 250-mm Whatman Partisil-10 PAC column, 12:12:1 hexane–tetrahydrofuran–methanol, detector at 600 nm.

2 H). These signals are due to the para, meta, and ortho protons, respectively, of a phenyl ring, as confirmed by the spin decoupling experiments in Figure 2. The extraordinary high field position of these phenyl protons, which requires their placement in the porphyrin ring current, and the presence of all the protons due to protoporphyrin IX in the NMR spectrum uniquely identify the green porphyrin as *N*-phenylprotoporphyrin IX (dimethyl ester). Very similar, if simpler, spectra are obtained if the pigment is first resolved into two fractions. The NMR spectra of the two fractions, however, indicate that each fraction consists of two very similar isomeric structures. The four possible isomers of *N*-phenylprotoporphyrin thus appear to be formed, the isomers with the *N*-phenyl substituent on two of the nonequivalent nitrogen atoms being resolved from those with the substituent on the other two nitrogens. Studies in progress have established that the green porphyrin mixture is a major product of the phenylhydrazine–hemoglobin interaction.

The globin envelope does not play an essential role in arylation of heme by phenylhydrazine. In effect, a similar mixture of *N*-phenylprotoporphyrin IX isomers is produced in moderate yield when hemin is allowed to react with phenylhydrazine·HCl.[9] The reaction does not take place under anaerobic conditions. This reaction not only excludes a mechanistic role for the protein in heme arylation, it also confirms the structure of the green porphyrin and provides the first synthetic route to *N*-arylporphyrins. The scope of the synthetic procedure is under investigation.

Benzene, nitrogen, hydrogen peroxide, and superoxide are known products of the aerobic reaction of phenylhydrazine with hemoglobin.[3,10] Phenyl radicals generated by the heterolysis of phenyldiazene are presumed as intermediates in benzene formation.[11] The reaction of phenyl radicals with prosthetic heme thus offers an attractive mechanism for the biological formation of *N*-phenylheme, although the details of the interaction and its role in hemoglobin precipitation and cell lysis remain to be elucidated.[12] The only precedent for this process is our recent finding that the prosthetic heme of cytochrome P-450 is *N*-alkylated during catalytic turnover of olefinic and acetylenic substrates.[14] The involvement of the heme nitrogens in the interactions of both hemoglobin and cytochrome P-450 suggests a possible general role for such reactions in abnormal heme catabolism.

Acknowledgment. This research was supported by NIH Grants GM-25515, P50 AM-18520, and RR-00719. P.R.O.M. is a Fellow of the Alfred P. Sloan Foundation (1978–1982).

(8) Porphyrin ring NMR assignments: 9.90–10.25 (4 H, meso), 7.60–8.40 (2 H, $CH{=}CH_2$), 5.90–6.50 (4 H, $CH{=}CH_2$), 3.95–4.40 (4 H, $ArCH_2$) 3.25–3.70 (18 H, CH_3), and 2.70–3.30 ppm (4 H, CH_2CO_2).

(9) Hemin chloride (20 mg) and disodium EDTA (10 mg), dissolved in 2 mL of NaOH (0.1 N) and diluted to 10 mL with H_2O, were brought to pH 8 with phosphate buffer (1 N) before phenylhydrazine·HCl (50 mg) was added. After 1 h, the mixture was acidified (1 N HCl), and the precipitated solid was put through the same workup as the hemoglobin pigment.[5]

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(11) (a) Huang, P. C.; Kosower, E. M. *J. Am. Chem. Soc.* **1968**, *90*, 2367–2376. (b) Nicholson, J.; Cohen, S. G. *Ibid.* **1966**, *88*, 2247–2251.

(12) Oxyhemoglobin is converted to methemoglobin by reducing agents like phenylhydrazine[13a] and superoxide,[13b] a product of phenylhydrazine autoxidation.[10b] Methemoglobin in turn reverts to oxyhemoglobin on reaction with superoxide.[13b] The oxidation state of the heme during the arylation reaction is therefore uncertain at this time. An in-depth examination of the reaction mechanism is under way.

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Structure Revision of 37 Lycoctonine-Related Diterpenoid Alkaloids

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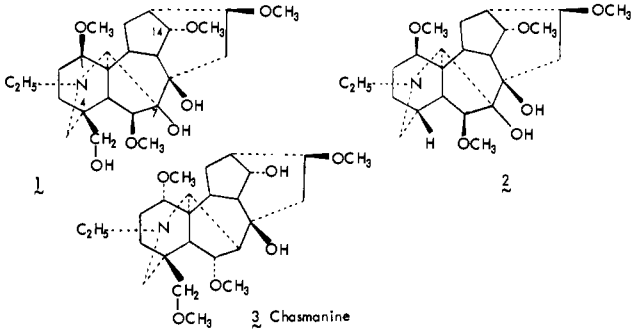
The structure of the key C_{19}-diterpenoid alkaloid, lycoctonine, was established as **1** in 1956 by an X-ray analysis of the hydroiodide salt of 4-de(oxymethylene)lycoctonine (**2**).[1] Since then



the structures of most of the lycoctonine-type alkaloids[2] have been assigned on the basis of chemical and/or spectral correlation with lycoctonine.[3] In 1976, we revised[4] the configuration of the C-(1)-methoxyl group of chasmanine (**3**) from β to α on the basis of X-ray analysis and mentioned that the reported[5] chemical correlation between browniine and chasmanine is in error. This statement assumed that the structure of browniine was correct, since its structure had been assigned[6] on the basis of direct chemical correlation with lycoctonine. Recently, we learned that X-ray analysis of two rearrangement products of a lycoctonine derivative has indicated a configuration of the C(1)-methoxyl group in these two compounds opposite to that reported in the original X-ray analysis of compound **2**.[7] This development has prompted us to submit a preliminary report of our own investigation of this problem.

Methylation of delsoline (**4**) with methyl iodide and sodium hydride in a sealed tube at reflux temperature afforded mainly the known alkaloid delphatine (**5**).[8,9] Because the α configuration of the C(1)-hydroxyl in delsoline is well established by chemical[10]

(1) M. Przybylska and L. Marion, *Can. J. Chem.*, **34**, 185 (1956); **37**, 1843 (1959).

(2) These alkaloids possess the skeleton of lycoctonine in which the C(7) position is always oxygenated by hydroxyl or methoxyl or methylenedioxyl groups.

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(4) S. W. Pelletier, W. H. DeCamp, and Z. Djarmati, *J. Chem. Soc., Chem. Commun.* 253 (1976).

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(7) O. E. Edwards, personal communication to S. W. Pelletier, 23 March 1981, O. E. Edwards, *Can. J. Chem.*, in press; M. Cygler, M. Przybylska, and O. E. Edwards, *Acta Crystallogr.* in press.

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(9) S. W. Pelletier, R. S. Sawhney, H. K. Desai, and N. V. Mody, *J. Nat. Prod.*, **43**, 395 (1980).

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