Methyl 10-Epipheophorbide a: an Unusual Epimeric Stability Relative to Chlorophyll a or a'[1]

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Methyl 10-epipheophorbide a has been isolated from the equilibrium mixture of **1** and **2** by column chromatography on powdered cellulose (Whatman CF-1) using 10% ethylene dichloride in hexane as the eluting solvent. The equilibrium mixture of **1** and **2** contains 13–15% **2** compared to 15–20% chlorophyll a' present in the equilibrium mixture of chlorophyll a and a'. The rate of epimerization of **2** to **1** is much slower than the rate of chlorophyll a' to a conversion.

We report the isolation of methyl 10-epipheophorbide a (**2**) and its slow rate of epimerization relative to either chlorophyll a or a'. The stability of a pheophorbide analogue that is epimeric at the chiral C-10 position is particularly interesting since this chiral center may play an important role in both the synthesis and the photosynthetic activity of chlorophyll.[2] Several models have been proposed for the structure of the photosynthetic reaction center involving a chlorophyll dimer,[3] and the integrity of C-10 has been demonstrated as a structural requirement for the biological activity of chlorophyllase.[4]

Reaction-center chlorophyll makes up only a small fraction of the total leaf chlorophyll,[5] and until recently it has been very difficult to determine with reliability whether a minor component found in a plant leaf workup was an artifact or an important natural product occurring in trace amounts. Recent advances in instrumental studies have aided in the structural identification of these reactive species present in small amounts.[6,7] In bacterial systems capable of undergoing photosynthesis, a metal-free form of bacteriochlorophyll has been accepted as being part of the bacterial photosynthetic reaction center.[8] Evidence for or against the involvement of a metal-free chlorin in green plant photosynthesis is still forthcoming.

Results and Discussion

When a sample of **1** (Figure 1), isolated as described in the Experimental Section, was chromatographed on powdered cellulose, thin layer chromatography of the early fractions clearly showed two closely related constituents. The 220-MHz ^{1}H NMR spectrum of the early fractions (Figure 2) also showed the presence of two very similar chlorins. The α, β, and δ-methine proton resonances which are singlets in the ^{1}H NMR spectrum of pure **1**[9] appear as six peaks between 8.4 and 9.5 ppm. Examination of the low-field methyl region between 3.0 and 4.7 ppm further corroborated this conclusion. Instead of the 5 singlet resonances expected, 9 peaks are observed.

Further examination of the ^{1}H NMR spectrum eliminated several plausible contaminants. The AB lines from the vinyl ABX system together with the C-10 proton resonances (at 6 ppm) integrated for three protons, ruling out both a mesopheophorbide contaminant as well as any compound resulting from the loss of the active hydrogen at C-10. The integrity of the low-field methyl groups and the methine bridge protons eliminated a variety of other possible contaminants.

The small difference in chemical shift observed for the protons affected suggested the subtle differences that would be expected from diasteriomers. Compound **1** has three chiral centers at C-7, C-8, and C-10. When ring V is cleaved with methylamine,[10] as in Scheme I, isochlorin e_4-6-carboxymethylamide dimethyl ester (**3**, Figure 3), in which C-10 is no longer chiral, is formed. If the mixture were epimeric at either C-7 or C-8 one would expect to see continued evidence in subsequent ^{1}H NMR spectra. When Scheme I was performed

Scheme I

$$1 + 2 + CH_3NH_2 \rightarrow 3$$

with a quantitative workup, the resulting ^{1}H NMR spectrum demonstrated the presence of only one chlorin. A similar multiplicity of peaks in the ^{1}H NMR spectra of a mixture of chlorophyll a and a' has been previously reported.[11]

Once the composition of the chlorin mixture was established the unexpected stability of **2** became a point of interest. Katz et al.[11] have studied the epimerization of chlorophylls at C-10 in a variety of solvents. They determined that an equilibrium mixture contained 15–20% chlorophyll a' (10-epichlorophyll a), and that this equilibrium mixture was temperature independent over a temperature range of 30 to 70 °C. The half-life for the epimerization of chlorophyll a at C-10 in either pyridine or tetrahydrofuran was estimated to be about 2 h.[11] It has further been cited that chlorophyll a' epimerizes "quickly" at 10 °C in tetrahydrofuran.[11]

We have observed that **2**, a metal-free analogue of chlorophyll a, is stable indefinitely in $CDCl_3$ at room temperature. In the presence of 0.58 M deuteriopyridine, the half-life for epimerization is 45 h at 25 °C. When **2** was held at 56.5 °C in the presence of deuteriopyridine, it epimerized slowly to an equilibrium mixture containing 13–15% **2**. The half-life for this process at 56.5 °C is 2.5 h.[12]

We believe that the origin of the large difference in the rate of epimerization at the C-10 position is the result of a considerable conformational difference between the chlorophyll and pheophorbide. Although ring V in the chlorophyll introduces considerable distortion and the magnesium is not in the plane of the chlorin ring, the central metal ion does serve to keep the four porphyrin nitrogens more or less coplanar.[13] Proton NMR evidence suggests that ring III in the pheophorbides is tipped upward at quite a sharp angle with N–H 1.4 Å above the plane of the porphyrin ring.[14] This is a sufficient enough distortion to stop internal movement of the N–H protons and move the ring III N–H from δ −1.7 to 0.90.[14] This distortion would also be expected to lead to considerable relief

Table I. 220-MHz ^{1}H NMR of 1 and 2 at Infinite Dilution in $CDCl_3$

Proton	Chemical shift, ppm 1	2
β-Methine	9.49	9.45
α-Methine	9.34	9.30
δ-Methine	8.57	8.51
OCH_3 (C-10)	3.91	3.85
(C-7)	3.70	3.66
CH_3 (C-5)	3.60	3.60
(C-1)	3.40	3.39
(C-3)	3.20	3.18
N-H (pyrrole)	0.90	0.90
	1.52	1.73

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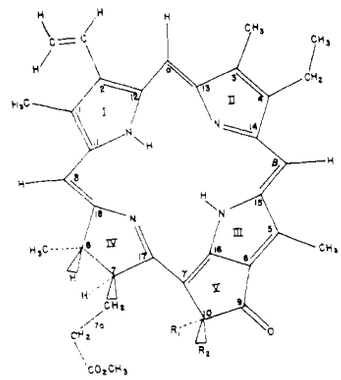

Figure 1. Structure of methyl pheophorbide a (**1**, $R_1 = CO_2CH_3$, R_2 = H) and methyl 10-epipheophorbide a (**2**, R_1 = H, $R_2 = CO_2CH$).

of strain at the C-10 position and is reflected in the slower rate of epimerization at that position.

The conformational differences between chlorophyll a and a′ and **1** and **2** are only reflected to a small degree in the equilibrium constant for the epimerization. The 15–20% chlorophyll a′ reported at equilibrium[11] compares favorably to the 13–15% **2** we observe at equilibrium. The mechanism for the epimerization most likely involves either the C-10 anion or the enol of the carbonyl group at C-9. The steric deformations imposed on the chlorophyll molecule by the incorporation of the Mg(II) ion affects the transition-state energy leading through these intermediates more than the ground-state energies reflected in the equilibrium constant and results in the more rapid epimerization of **2**. Chlorophyll a, **1**, and pheophytin a all exchange the C-10 proton under mild conditions. A careful study of the rate of C-10 proton exchange in each species as compared to the rate of epimerization in each species would be useful in understanding the mechanism of reaction.

It may also be noted that the chemical shift of protons in **2** on the outside of the porphyrin ring are to high field of **1**, while the N–H of **2** is to low field of **1** (Table I). This is also consistent with a distortion of the basic chlorin system, leading to a lower net ring-current effect in **2** than in **1**.

Experimental Section

Methyl Pheophorbide a (1). The procedure of Strain[15] was used to prepare chlorophyll a from fresh spinach leaves. The final sucrose column was replaced by Willstatter's procedures for the preparation[16a] and purification[16b] of methyl pheophorbide a. A final purification by column chromatography (described below) was added to remove any last traces of methyl pheophorbide b or allomerized methyl pheophorbide a. Inadvertently it also provided a fraction of **1** that was highly enriched in **2**.

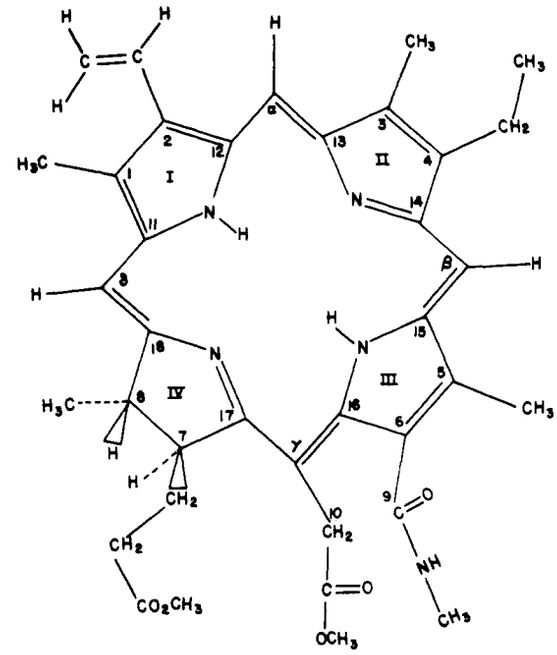

Figure 3. Structure of isochlorin e_4-6-carboxymethylamide dimethyl ester (**3**).

Methyl 10-Epipheophorbide a (2). A solution of **1** containing 0.546 g in a minimal amount of ethylene dichloride (EDC) was absorbed on 6 g of powdered cellulose (Whatman CF-1) and the EDC removed under vacuum, followed by a gentle stream of N_2 until the odor of EDC was no longer detectible. The chlorin sample was placed on a cellulose column (5.0 × 24.5 cm) packed in hexane, and the column was developed with 10% ethylene dichloride in hexane. The initial 25% of the total chlorin eluted from the column was enriched in **2** with individual fractions containing 80% **2**. TLC on Eastman Chromagram silica gel sheets, using 6% acetone in CCl_4 separated the epimers for analytical determination (R_f: **1**, 0.20; **2**, 0.23).

Isochlorin e_4-6-Carboxymethylamide Dimethyl Ester (3). The following procedure was found to be superior to those described by Fischer[17] and Pennington.[10] To a suspension of **1** (1.20 g, 1.98 mmol) in 10 mL of peroxide-free[18] tetrahydrofuran a solution containing 2 mL of 40% aqueous CH_3NH_2 (23.1 mmol) and 8 mL of THF was added dropwise with rapid stirring. After 2 h under N_2, the green

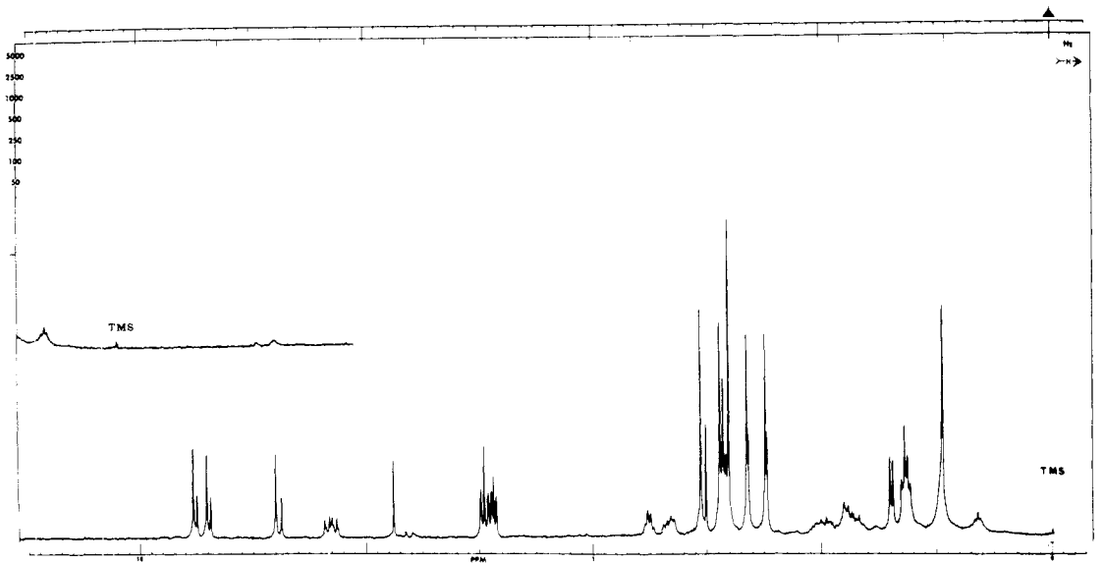

Figure 2. The 220-MHz ^1H NMR spectrum of a sample containing 67% **1** and 33% **2**.

solution was transferred to Et_2O (150 mL), washed with H_2O (100 mL × 4), and extracted with cold 5% HCl (50 mL × 6). The acid extract was washed with Et_2O (100 mL × 2) and extracted with CH_2Cl_2 (50 mL × 4). The CH_2Cl_2 extract was washed with H_2O and the solvent removed; yield after vacuum drying, 1.07 g (85%). Workup of the ether phases yielded 0.131 g (10.4%) of impure **2.** The major fraction was chromatographed on a silicic acid column (2.5 × 32 cm) with methanol/acetone/CCl_4 1:20:79 and twice crystalized from methylene chloride/pentane. Anal. Calcd for $C_{37}H_{43}O_5N_5$: C, 69.67; H, 6.81; N, 10.98. Found: C, 69.31; H, 6.73; N, 11.11.

To verify the structure of **2** the procedure was modified to employ a quantitative workup. $CHCl_3$ was added directly to the reaction flask, and the organic phase was washed with successive portions of cold water, removing the excess CH_3NH_2 but no chlorin. After solvent removal and vacuum drying, the entire reaction was analyzed by ^1H NMR, demonstrating the presence of only one chlorin.

Determination of the Epimer Ratio. In addition to the C-10 carbomethoxymethyl group used by Katz et al.,[11] the resonances for the β- and δ-methine protons were selected on the basis of minimum signal interference and maximum epimer chemical-shift difference. Either multiple scans were averaged on a Varian A-60 spectrometer and integrated, or in the case of very dilute samples the FT 220-MHz ^1H NMR spectrum was obtained and the peaks were integrated to determine the epimer ratio.

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Registry No.—**1,** 5594-30-9; **2,** 64070-09-3; **3,** 64045-79-0.

References and Notes

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An Improved Chemical Synthesis of Racemic Phycocyanobilin Dimethyl Ester[1]

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The title compound, a bile pigment-like product isolated from the photosynthetically active chromoproteins of the blue-green and red algae, has been synthesized chemically in 32% overall yield from readily accessible starting materials. The key reaction of the synthesis consists of the preparation of the 3,4-dihydro-5(1*H*)-pyrromethenone **11a** by condensation of the monothiosuccinimide **8** with the pyrrole derivative **10.** This reaction represents a new type of formation of C=C bonds using resonance-stabilized phosphorus ylides.

Phycocyanobilin is the blue pigment released by boiling methanol from the photosynthetically active chromoproteins R and C phycocyanin and allophycocyanin of the blue-green and red algae.[2,3] The structure of phycocyanobilin has been elucidated by means of spectroscopic[4,5] as well as degradation studies.[6]

Three years ago a convergent chemical synthesis of racemic phycocyanobilin dimethyl ester (***rac*-15**) was achieved for the first time in our laboratory by condensation of methyl 5′-formylisoneoxanthobilirubinate (**13b**) with the 5(1*H*)-pyrromethenone derivative **11b.** The latter was obtained by reaction of the substituted monothiosuccinimide **8** with the brominated pyrrole derivative **9** under the conditions of Eschenmoser's sulfide contraction method.[7] However, the overall yield of this synthesis amounted only to 0.6% when referred to 3-ethylidene-4-methylpyrrolidine-2,5-dione which was used as a precursor of the ring I of the bile pigment. This unsatisfactory result was attributable to the occurence of several critical steps in the synthesis, namely: (i) the nonregiospecific transformation of the 3-ethylidene-4-methylpyrrolidine-2,5-dione into the corresponding 2-monothio derivative **8,** (ii) the moderate yield of the sulfide contraction reaction of **8** with **9** even though it could be improved from 18 to 49% by carrying out the reaction at −78 °C, and (iii) the relatively low reactivity of the *tert*-butyl ester **11b** toward the aldehyde **13b.**

We have now improved considerably the overall yield of the synthesis of phycocyanobilin dimethyl ester by introducing some substantial modifications into our earlier approach. Thus, monothioimide **8** was prepared regiospecifically as follows: alkylation of diethyl cyanomethylphosphonate (**1**)[8]

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