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# Bacteriochlorophylls *c* from *Chloropseudomonas ethylicum*. Composition and NMR Studies of the Pheophorbides and Derivatives

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**Abstract:** The carbon-13 and proton NMR spectra of the methyl pheophorbides **6**, methyl 2-vinylpheophorbides **2**, methyl mesopheophorbides **7**, and other degradation products from the green sulfur bacterium *Chloropseudomonas ethylicum* are described and assigned. In order to clarify certain spectra, model chlorins substituted with *n*-propyl, isobutyl, propenyl, and isobutenyl side chains are synthesized from the chlorophyll *b* degradation product, rhodin $g_7$ trimethyl ester (**17**). Successful separations of the homologous mixture of pheophorbides, using reverse-phase high-performance liquid chromatography, are detailed; only four major bands (rather than the six obtained from *Chlorobium thiosulfatophilum* cultures) were observed, and only a minute amount of 4-ethyl-5-methylpheophorbide (band 6) was apparent in the *C. ethylicum* culture presently being studied.

The bacteriochlorophylls *c* (*Chlorobium* chlorophylls 660) from various green sulfur bacteria (Chlorobiaceae) have been extensively investigated. The structures shown in **1** have been assigned to the homologues in the mixture of bands, but those assigned to bands 1 and 3 (**1a** and **1c**, respectively) must be regarded as tenuous, since there exists no positive evidence for $\delta$ meso groups other than methyl.[2] Recently, Brockmann has described the isolation of a series of 3-formylchlorophylls (bacteriochlorophylls *e*)[3] and chlorophylls with esterifying alcohols other than farnesol,[4] and, again, in no case was a $\delta$ meso ethyl substituent observed.

In earlier papers[2b,5] we described initial studies on the biosynthesis of the *Chlorobium* chlorophylls from *Chloropseudomonas ethylicum*,[6] and several novel chemical transformations of these chlorophylls were described. In particular, a unique vinyl cyclization (**2** → **3**) and a photooxidative ring opening (**4** → **5**) were characterized. The 2 + 2 addition of oxygen to a macrocyclic double bond, which we proposed,[2b] has recently been confirmed by Risch,[7] and by ourselves, and we have carried out experiments with chlorin models that indicate that the specificity of the oxidative cleavage, which gives only one photobilin isomer, **5**, is controlled by the presence of the cyclopentanone ring.[8] In the present paper we describe the mass and NMR spectra of the pheophorbides and derivatives from *Chloropseudomonas ethylicum*,[6] and describe our ultimately successful attempts at separation of the homologous mixture. Our previous NMR assignments[2b] of the *n*-propyl and isobutyl groups had depended upon comparisons with *n*-propyl- and isobutylbenzenes. Owing to the fact that chlorophylls and porphyrins (unlike simple benzenoid compounds) undergo extensive aggregation phenomena[9] which affect observed chemical shifts, we also describe the partial syntheses of *n*-propyl and isobutyl derivatives (from pheophytin *b*) which enable a better estimation of the NMR parameters of these substituents in the homologous mixture.

## Field Desorption Mass Spectra

Using *Chlorobium thiosulfatophilum*, Holt and co-work-

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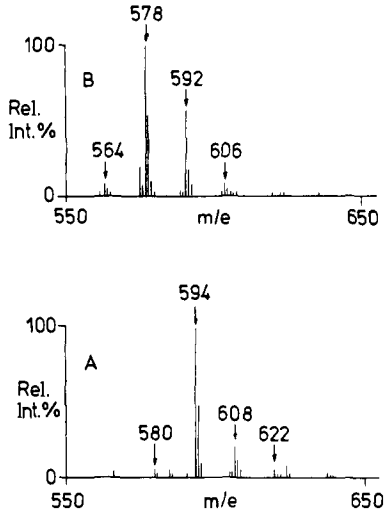
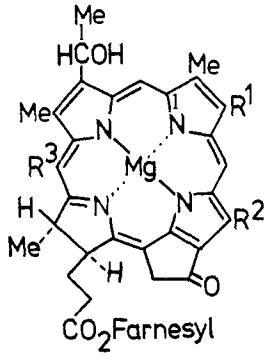



**Figure 1.** Field desorption mass spectra (high mass region) of the homologous pheophorbide mixture from *C. ethylicum:* (A) methyl pheophorbides **6**; (B) methyl mesopheophorbides **7**.

ers[10] carried out structural studies and arrived at the six homologues given in **1**. On the other hand, Brockmann,[11] using

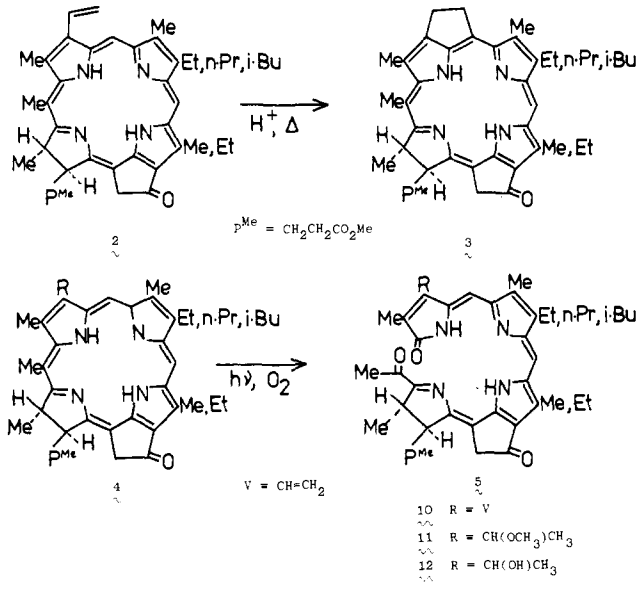



**1**

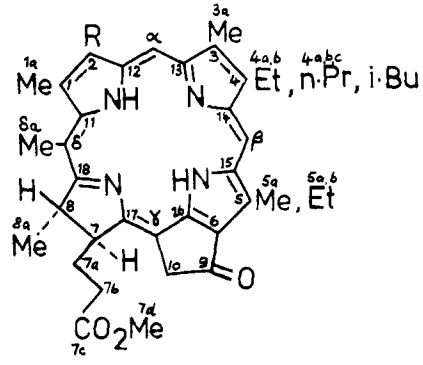
| Fraction or Band | $R^1$ | $R^2$ | $R^3$ | Percentage of Mixture (from Ref. 10) |
|---|---|---|---|---|
| 1 (Cmpd 1a) | i-Bu | Et | Et | 0.5% |
| 2 (Cmpd 1b) | i-Bu | Et | Me | 0.5% |
| 3 (Cmpd 1c) | n-Pr | Et | Et | 2.0% |
| 4 (Cmpd 1d) | n-Pr | Et | Me | 16.0% |
| 5 (Cmpd 1e) | Et | Et | Me | 71.0% |
| 6 (Cmpd 1f) | Et | Me | Me | 10.0% |

*Chlorobium limicola*,[6] reported that no 5-methyl homologues were present in his extracts. Electron impact mass spectroscopic studies of our extracts from *C. ethylicum* suggested that only four components were present. However, the spectra were somewhat complicated, so we turned to field desorption mass spectrometry, which usually shows only molecular ions.

Figure 1A shows the field desorption spectrum for the methyl pheophorbides **6**. The peak at *m/e* 594 corresponds to fraction 5, the major homologue. Fractions 6, 4, and 2 can be seen at *m/e* 580, 608, and 622, respectively. A molecular ion for fraction 1 is not present. Whether this is due to its nonexistence or the fact that it is only present in a minute amount is impossible to say at this stage. Fraction 3 has the same molecular weight as fraction 2 and so again nothing can be deduced at this point. A low intensity molecular ion at *m/e* 576 is due to dehydration of the 2-hydroxyethyl function of fraction 5. Figure 1B shows the spectra of the methyl mesopheophorbides **7** from *C. ethylicum* and again only fractions 6, 5, 4, and




2 are evident. The methyl mesopheophorbides **7** were derived[5] from the methyl pheophorbides **6** by dehydration of the 2-




**6** R = $CH(OH)CH_3$

**7** R = Et

**8** R = $CH(OCH_3)CH_3$

**9** R = $CH(OCH_2CH_3)CH_3$ (ethyl ester)

hydroxyethyl group to a vinyl group and then hydrogenation to an ethyl substituent, resulting in an overall decrease of 16 in the molecular weights of the homologues. One interesting feature of both Figures 1A and 1B is that, although fraction 6 represented 10% of the total mixture from *Chlorobium thiosulfatophilum*,[10] judging solely from the intensity of the molecular ion in both spectra it does not appear to be more than 5% of the mixture in *C. ethylicum*.

## NMR Spectra

**Carbon-13 NMR Spectra.** From the mass spectral studies it appeared that, within the mixture of homologues, fraction 5 accounted for about 70% of the material. (See later for separation data.) It therefore seemed unlikely that $^{13}C$ spectra of such derivatives would show significant resonances other than those derived from fraction 5. Examination of the $^{13}C$ spectra of methyl pheophorbides **6**, methyl 2-vinylpheophorbides **2**, and methyl mesopheophorbides **7**, obtained by degradation of the chlorophylls from *C. ethylicum*, confirmed the structure proposed for fraction 5 by Holt et al.[10] Peaks of much lower intensity than those assigned to fraction 5 were also present due to the other homologues.

The nomenclature used is shown in structure **6**. Table I shows the $^{13}C$ chemical shifts and assignments in $CDCl_3$ and TFA solution of the pheophorbides **6, 2, 7, 3,** and also the

**Table I.** $^{13}C$ NMR Spectra[a-c] of Methyl Pheophorbides **6**, Methyl 2-Vinylpheophorbides **2**, Methyl Mesopheophorbides **7**, Methyl 2,$\alpha$-Ethylenepheophorbides **3**, and Methyl 2-Methoxyethylpheophorbides **8** from *Chloropseudomonas ethylicum*[d]

| assigned carbon resonances | **6** | pheophorbide from **1d**[d] | **2** | **7** | **7**[c] | **3** | **8** |
|---|---|---|---|---|---|---|---|
| 1a | 16.3 | 16.5 | 17.4*[f] | 16.3 | (14.5) | (16.5) | 16.3 |
| 2a | 65.1 | 65.4 | 129.8 | 19.3* | 18.7 | (23.5) | 74.6 |
| 2b | 25.5 | 25.6 | 123.8 | 16.7* | (16.4) | (38.4) | 24.6 |
| 3a | 11.1 | 11.4 | 11.1 | 11.1 | 11.3 | 13.3 | 11.3 |
| 4a | 19.3 | 28.1 | 19.4 | 19.3* | 21.0 | 19.1 | 19.5 |
| 4b | 17.3 | 17.3 | 17.4* | 17.3 | (16.5)* | 17.5 | 17.4 |
| 4c | | 14.4 | | | | | |
| 5a | 20.5 | 20.5 | 20.6 | 20.4 | 22.9 | 20.6 | 20.6 |
| 5b | 16.6 | 16.7 | 16.7 | 16.7* | (16.5)* | (16.8) | 16.8 |
| 7 | 51.5* | 51.6* | 51.8 | 51.3* | 53.4 | 52.1 | 51.5 |
| 7a | 30.9 | 31.0 | 31.0 | 30.8 | 32.2 | 31.0 | 30.9 |
| 7b | 29.9 | 30.0 | 30.0 | 29.9 | 29.8 | 29.7 | 30.0 |
| 7c | 173.2 | 173.2 | 173.2 | 172.6 | 178.7 | 173.3 | 173.2 |
| 7d | 51.5* | 51.6* | 51.5 | 51.3* | 54.6 | 51.5 | 51.6 |
| 8 | 48.1 | 48.2 | 48.3 | 48.2 | 46.6 | 47.7 | 48.4 |
| 8a | 20.7 | 20.8 | 20.9 | 20.8 | 20.2* | 21.2 | 20.9 |
| 9 | 195.5 | 195.6 | 195.4 | 194.9 | 198.7 | 195.9 | 195.5 |
| 10 | 48.6 | 48.6 | 48.8 | 48.5 | 49.1 | 48.7 | 48.8 |
| $\alpha$ | 97.2 | 97.3 | 97.3 | 95.1 | 105.8 | (103.9) | 97.1 |
| $\beta$ | 102.4 | 102.7 | 102.6 | 102.3 | 111.4 | 102.7 | 102.6 |
| $\gamma$ | 105.5* | 105.6* | 105.8* | 105.3 | 105.4* | (104.5)* | 105.7* |
| $\delta$ | 105.5* | 105.6* | 105.8* | 105.0 | 105.4* | (104.5)* | 105.7* |
| $\delta$a | 20.1 | 20.3 | 20.2 | 20.0 | 20.2* | 18.5 | 20.4 |
| 2a-$OCH_3$ | | | | | | | 57.1 |
| centers of $CDCl_3$ or TFA | 77.0 | 76.9 | 76.9 | 76.7 | 115.9<br>162.7 | 76.9 | 76.9 |
| unassigned carbon resonances, signal no. | | | | | | | |
| 1 | 130.2 | 130.3 | 130.5 | 129.8 | 134.5 | 120.1[e] | 130.5 |
| 2 | 131.1 | 131.2 | 131.0 | 130.4 | 135.4 | 122.2 | 132.4 |
| 3 | 132.7 | 132.7 | 133.7 | 133.9 | 139.4 | 130.8 | 133.2 |
| 4 | 135.3 | 135.4 | 135.5 | 134.5 | 140.5 | 133.9 | 135.6 |
| 5 | 136.3 | 137.1 | 136.2 | 135.3 | 141.6 | 136.4 | 136.5 |
| 6 | 137.9 | 137.9 | 138.1 | 137.3 | 142.1 | 138.8 | 137.2 |
| 7 | 140.3 | 140.3 | 138.9 | 141.0 | 144.3 | 140.0 | 140.5 |
| 8 | 142.2 | 142.3* | 140.7 | 142.8 | 147.4 | 145.1 | 142.4 |
| 9 | 143.9 | 142.3* | 144.1 | 143.7 | 149.0 | 147.8 | 144.1 |
| 10 | 147.6 | 147.6 | 147.8 | 147.4 | 150.7 | 148.1 | 147.8 |
| 11 | 151.2 | 151.8 | 151.2 | 150.4 | 151.2 | 148.7 | 151.5 |
| 12 | 152.8 | 152.7 | 153.1 | 152.7 | 151.4 | 152.2 | 153.0 |
| 13 | 158.7 | 158.8 | 158.8 | 158.0 | 156.2 | 158.4 | 158.9 |
| 14 | 172.1 | 172.1 | 172.2 | 172.0 | 158.4 | 170.8 | 172.3 |

[a] Chemical shifts are in parts per million downfield from tetramethylsilane used as internal standard. [b] Multiplicity of resonances in proton off-resonance decoupled spectra was compatible with all assignments. [c] All spectra were run in $CDCl_3$, except for compound **7** which was also run in trifluoroacetic acid (TFA). [d] The methyl pheophorbides from band 4 (**1d**) were obtained, courtesy of Drs. Holt and Wasley, from *Chlorobium thiosulfatophilum*. [e] Additional signal present at 150.1 ppm. [f] Asterisks denote coincident resonances; signals in parentheses are ambiguous; signals underlined were almost absent in the spectrum of the deuterioacetic acid exchanged sample (see text).

methyl 2-methoxyethyl derivatives **8**, vide infra. The spectrum of the methyl pheophorbide from fraction 4 (*Chlorobium thiosulfatophilum*) is also included in Table I.

**(a) Meso Carbon Resonances.** There has been some argument concerning the position of the novel meso methyl group in the bacteriochlorophylls *c*, though the $\delta$ position was strongly favored by synthetic and degradative work.[2] The downfield shift of the $\alpha$ and $\beta$ meso carbon resonances of chlorins in TFA relative to $CDCl_3$ solution[12] was applied as a diagnostic effect to identify the site of meso methylation in the methyl mesopheophorbides. Substantial downfield shifts of the two methine carbons in TFA solution confirmed them as $\alpha$ and $\beta$ meso carbons, proving that the methyl substitution is at the $\delta$ position in fraction 5 of bacteriochlorophyll *c* and therefore in all of the fractions.

The high-field meso carbon resonance is assigned to the $\alpha$-methine carbon, because of its comparable chemical shift to the $\alpha$-C of methyl pyropheophorbide *a*, and also because the conversion of 2-vinyl to 2-ethyl, i.e., methyl 2-vinylpheophorbides to methyl mesopheophorbides, results in a significant 2-ppm upfield shift of this signal.[12] The $\beta$ carbon resonates slightly upfield of the equivalent signal in methyl pyropheophorbide *a* (ca. 0.5 ppm). The $\gamma$ and $\delta$ carbons are coincident in the 2-hydroxyethyl-**6** and 2-vinyl-**7** derivatives; the assignment of the $\gamma$-C to the lowest field meso resonance in the 2-ethyl derivative **7** is based on our earlier work.[12]

The argument concerning the position of meso methylation was caused by the phenomenon of bacterochlorophylls *c* and derivatives undergoing deuterium exchange at one of their two unsubstituted meso positions. To locate the site of this deuteration, methyl mesopheophorbides **7** were heated to 110 °C with deuterioacetic acid. The compound underwent deuterium exchange at one of the meso hydrogens and at the C-10 methylene group. The high-field methine resonance in the $^{13}C$

**Table II.** Quaternary Carbon Assignments of the $^{13}C$ NMR Spectra of Methyl Pheophorbide *a* (**25**); INDOR Spectra from Certain Proton Resonances[a]

| proton | INDOR lines[b] | original assignments[a] | reassignments |
|---|---|---|---|
| 5a | 128.6, 137.7 | C-5, C-15 | C-5 and ? C-6, C-15 |
| 10 | 150.2, 160.8, 169.6 | C-16, C-6, C-10a | C-16, C-17, C-10a |
| 10b | 169.6 | C-10a | C-10a |
| 7a | 173.5 | C-17 and C-7c | C-7c |
| 7d | 173.2 | C-7c | C-7c |

[a] Reference 14. [b] Shifts in parts per million downfield from tetramethylsilane.

spectrum, assigned to the $\alpha$-C, was greatly reduced in intensity in the spectrum of the deuterated sample, as was the high-field peak in the proton spectrum.

**(b) Substituent Carbon Resonances.** The assignments of C-3a, -7a, -7b, -7c, -7d, -9, and the vinylic carbons of the 2-vinyl derivative were accomplished by direct comparison with chlorophyll *a* degradation products.[12] The methylene C-10 was unequivocally assigned from the specific deuteration outlined above. Methine C-7 resonates very close to the comparable signal in methyl pyropheophorbide *a*; however, C-8 experiences an upfield shift of ca. 1.5 ppm compared with the same compound. This, in effect, confirms the original assignments of C-7 and -8 in methyl pheophorbide *a*, as a significant perturbation at C-8 due to the adjacent $\delta$ methyl group would be expected. A similar perturbation should occur at the methyl C-1a; in fact, a downfield shift relative to methyl pyropheophorbide *a* of 5.6 ppm occurs in the 2-vinyl derivative **2**. This assignment was confirmed by an upfield shift of 1.1 ppm apparent in the conversion of methyl 2-vinylpheophorbides to methyl mesopheophorbides, due to an increase in steric compression. The methylene C-5a was assigned to the lower field of the methylene ethyl carbons on account of the adjacent $sp^2$ carbonyl, and C-4a to the higher field one. The low-field doublet was assigned to C-2a in the 2-hydroxyethyl derivative **6**, and the loss of the signal at 25.5 ppm on dehydration of methyl pheophorbides to methyl 2-vinylpheophorbides **2** led to its assignment as C-2b.

Examination of the $^{13}C$ spectrum of the methyl pheophorbide prepared by diazomethane treatment of the pheophorbide, fraction 4, from *Chlorobium thiosulfatophilum* (donated by Drs. A. S. Holt and J. W. F. Wasley), confirmed the structure proposed by Holt et al.[10] and aided in the differentiation between methyl C-4b and -5b of degradation products from *C. ethylicum*. The absence of a signal at 17.3 ppm in the methyl pheophorbide, fraction 4, results in the assignment of the lower field signal to the ethyl methyl carbon of ring B, i.e., C-4b. This concurs with the assignment of the equivalent carbon in meso derivatives from chlorophyll *a*.[12] Thus, it appears that the methyl carbons of ethyl substituents in rings A and C resonate at higher field than those in ring B. This would seem to fit the idea of rings A and C being electronically similar and unlike ring B, because they are flanked by a reduced ring and a "pyrrole" ring, thus causing a fundamental difference in shift between comparable substituents.

No satisfactory distinction between methyl C-8a and -$\delta$a was apparent, but the biosynthetic derivation of one of these signals was shown to be L-methionine,[5] and this must therefore be C-$\delta$a.

**(c) Macrocyclic Resonances.** There were too many variables to allow assignments of more than a few carbons. The lowest field resonances at 158 and 172 ppm were assigned to C-17 and -18 by analogy with derivatives of chlorophyll *a*. Comparison of the 4-ethyl-2-hydroxyethyl derivative, fraction 5, with the

**Table III.** Quaternary Carbon Assignments of the $^{13}C$ NMR Spectra of Methyl Pheophorbide *a* (**25**); Assignments of the FT Spectrum

| C | assigned resonance[a,b] | reassigned resonance[a] using Table II and ref 12 | assigned resonance[a,d,f] | C[c] |
|---|---|---|---|---|
| 1 | 131.6 | 131.1 | 131.8 | 2 |
| 2 | 136.3 | (135.7)* | 136.5 | 3 |
| 3 | 135.9*[e] | (135.7)* | 136.2 | 7 |
| 4 | 144.9 | 144.2 | 145.1 | 8 |
| 5 | 128.8* | 128.3* | 129.0* | 12 |
| 6 | 161.2 | 128.3* | 129.0* | 13 |
| 11 | 141.9 | 141.3 | 142.0 | 1 |
| 12 | 135.9* | (135.3) | 136.2 | 4 |
| 13 | 155.3 | 155.0 | 155.6 | 6 |
| 14 | 150.7 | 150.0 | 151.0 | 9 |
| 15 | 137.8 | 137.2 | 138.0 | 11 |
| 16 | 149.6 | 149.0 | 149.7 | 14 |
| 17 | 173.3* | 160.5 | 161.2 | 16 |
| 18 | 172.0 | 171.4 | 172.2 | 19 |

[a] Chemical shifts in parts per million downfield from tetramethylsilane. [b] Reference 14. [c] Nomenclature as used by Wray et al. (Wray, V.; Jürgens, U.; Brockmann, Jr., H. *Tetrahedron* **1979**, *35*, 2275–2283), corresponding to carbon atoms in column 1. [d] See Wray et al., footnote *c*. [e] Asterisks denote coincident resonances. Signals in parentheses are ambiguous. [f] NOTE ADDED IN PROOF. Since submission and review of the present paper, Wray et al. (see footnote *c*) have reported their assignments of the quaternary carbons in methyl pheophorbide *a* (**25**) and other chlorins. They claim to have *corrected* assignments in our earlier paper (ref 12) and give a list of assignments supposedly due to us, but which does not appear in our paper.[12] These corrected assignments are in fact identical with our own (in ref 12 and in: Unsworth, J. F. Ph.D. Thesis, University of Liverpool, Liverpool, 1975) and the confusion has arisen because of differing nomenclature systems used by the two different groups. To clarify the situation with regard to the claims of Wray et al., we have added columns four and five to this table at proof stage.

4-*n*-propyl-2-hydroxyethyl derivative, fraction 4, reveals downfield shifts of 0.8 and 0.6 ppm for signals 5 and 11, respectively, and an upfield shift of 1.6 ppm for signal 9, while the other macrocyclic resonances remain constant. These three signals must originate in ring B and are tentatively assigned as C-3 (136.3), C-4 (143.9), and C-14 (151.2) for the fraction 5 methyl pheophorbide. Comparison of the 2-hydroxyethyl derivative with the 2-vinyl derivative reveals a downfield shift of 1.0 ppm for signal 3 and upfield shifts of either one or both of signals 7 and 8; thus, signal 3 and either one or both of signals 7 and 8 must originate in ring A. Comparison of the spectrum of methyl mesopheophorbides **7** with either the 2-hydroxyethyl compound **6** or the 2-vinyl compound **2** reveals a number of changes in the macrocyclic region. Comparison of methyl pyropheophorbide *a* and its meso derivative with the equivalent bacteriochlorophyll *c* degradation products is also uninformative. Thus, there seems to be a greater perturbation for the transformation 2-vinyl into 2-ethyl in these degradation products than those derived[12] from chlorophylls *a* and *b* (compare also the high value of 2 ppm for the upfield shift for the $\alpha$ meso carbon in the same transformation), perhaps associated with different steric effects due to the $\delta$ methyl substituent.

The effect of the $\delta$-Br substituent in a mesobromopyropheophorbide mirrors that of the $\delta$-Me substituent in methyl pheophorbides from *C. ethylicum*. Methyl C-1a and -8a resonate at 18.3 and 20.8 ppm in the former case and at 16.3 and 20.7 ppm in the latter. In both cases C-1a experiences a substantial downfield shift (6.3 and 4.5 ppm, respectively) compared with methyl pyropheophorbide *a*, and C-8a experiences an upfield shift of 2 ppm. This confirms the comparison made by Lincoln et al.[13] of the effect of this $\delta$-bromine atom

**Table IV.** Assignments of Quaternary Carbons in Bacteriochlorophyll *c* Derivatives, and Comparison with Methyl Pyropheophorbide a[a]

| carbon | pheophorbide from **1d** **6** | **2** | **7** | methyl pyropheophorbide *a*[b] |
|---|---|---|---|---|
| 1 | 132.7 | 133.7 | 133.9 | 130.7 |
| 2 | 142.2 | 138.9 | 142.8 | (135.1) |
| 3 | 136.3 | 136.2 | 135.3 | (135.2) |
| 4 | 143.9 | 144.1 | 143.7 | 144.0 |
| 5 | 130.2 | 130.5 | 129.8 | 127.4 |
| 6 | 131.1 | 131.0 | 130.4 | 129.7 |
| 11 | 137.9 | 138.1 | 137.3 | 140.7 |
| 12 | 140.3 | 140.7 | 141.0 | (135.3) |
| 13 | 152.8 | 153.1 | 152.7 | 154.1 |
| 14 | 151.2 | 151.2 | 150.4 | 149.7 |
| 15 | 135.3 | 135.5 | 134.5 | 136.9 |
| 16 | 147.6 | 147.8 | 147.4 | 148.2 |
| 17 | 158.7 | 158.8 | 158.0 | 159.5 |
| 18 | 172.1 | 172.2 | 172.0 | 170.4 |

[a] Chemical shifts in parts per million downfield from tetramethylsilane. Spectra measured in $CDCl_3$. Assignments in parentheses are ambiguous. Main ambiguity problems still existing are between C-1 and C-6. [b] See ref 12.

with that of a methyl group, which has a similar van der Waals radius.

By using a modified heteronuclear INDOR technique, Boxer et al.[14] have assigned the quaternary carbons in the $^{13}C$ spectra of chlorophyll *a* and methyl pheophorbide *a*. In their analysis, the resonance at 173 ppm in methyl pheophorbide *a*, associated with C-7c, is considered to consist of two overlapping signals, the second of which is assigned to C-17. Methyl pyropheophorbide *a*, methyl mesopheophorbide *a*, and methyl mesopyropheophorbide *a* are structurally very similar to methyl pheophorbide *a*; furthermore, their $^{13}C$ NMR spectra coincide fairly closely.[12] Comparison of these spectra led to the consideration that the signal at 128 ppm consisted of three overlapping signals (C-2a and two macrocyclic carbons), thus differing from the interpretation of Katz et al.[14] Katz mentions difficulties in the assignments of C-6, -16, and -17, and his experimental data can be reinterpreted (Tables II and III) to give the assignments in Table IV.

**(d) Structural Confirmation of the Cyclized Methyl Pheophorbides 3.** The cyclized product originated from prolonged heating of the methyl pheophorbides **6** or methyl 2-vinylpheophorbide **2** in acid. The $^{13}C$ NMR spectrum revealed that the basic skeleton was unaltered and that the modification had only affected the shifts of C-3a, -2a, -2b and -δa among the substituent carbons. A downfield shift of 2 ppm for C-3a was compatible with an adjacent meso methylene group (cf. C-1a). A partially coupled spectrum revealed two triplets for the methylenes C-2a and -2b. The meso carbons showed only one unsubstituted carbon, which resonated as for a β meso carbon. Thus, the structure of this novel cyclized product was confirmed as **3**.

**Proton NMR Spectra.** Table V shows the $^1H$ NMR spectra of the methyl pheophorbides **6**, methyl 2-vinylpheophorbides **2**, methyl mesopheophorbides **7**, methyl 2-methoxyethylpheophorbides **8**, and ethyl 2-ethoxyethylpheophorbides **9**, from *C. ethylicum*. The derivatives **8** are artifacts of the methanol/sulfuric acid treatment of the chlorophylls, and are composed of a homologous mixture, like the methyl pheophorbides **6**, but each homologue is 14 mass units higher by mass spectrometry, indicating an extra methyl group. The polarity of the compound suggested that it no longer had a hydroxyl group, a fact borne out by the mass spectrum, which showed that the material had no tendency to dehydrate. It was thus deduced that the compound was the methyl 2-methoxyethylpheophorbide **8**. Further investigation showed that it was formed during the preparation of the methyl pheophorbides **6**, when the crude chlorophylls were treated with 5% sulfuric acid in methanol, but its formation was dependent upon the concentration of the acid. If 5% or more was used, the methyl pheophorbides **6** were produced, along with about 5% of the 2-vinyl derivative **2**, but if less than 5% acid in methanol was used, the methyl pheophorbides were again formed, this time accompanied by about 5% of the 2-methoxyethyl product **8**.

**Table V.** $^1H$ NMR Assignments of Methyl Pheophorbides **6**, Methyl 2-Vinylpheophorbides **2**, Methyl Mesopheophorbides **7**, Methyl 2,α-Ethylenepheophorbides **3**, Methyl 2-Methoxyethylpheophorbides **8**, and Ethyl 2-Ethoxyethylpheophorbides **9** from *Chloropseudomonas ethylicum*[a]

| proton | **6** | **2** | **7** | **3** | **8** | **9** |
|---|---|---|---|---|---|---|
| β-H | 9.49 | 9.45 | 9.45 | 9.24 | 9.52 | 9.52 |
| α-H | 9.86 | 9.37 | 9.28 | | 9.91 | 10.06 |
| 2a-H | 6.46 | 7.75 | 3.61 | 4.0–2.7 | 5.97 | 6.06 |
| 2b-H | | 7.05 | | | | |
| 10-$CH_2$ | 5.22 | 5.20 | 5.18 | 5.18 | 5.26 | 5.28 |
| 8-H | 4.57 | 4.50 | 4.40 | 4.40 | 4.60 | 4.61 |
| 7-H | 4.17 | 4.05 | 4.00 | 4.10 | 4.20 | 4.24 |
| 5a-$CH_2$ | 4.09 | 4.04 | 4.05 | 4.0–2.7 | 4.12 | 4.13 |
| δ-$CH_3$ | 3.86 | 3.79 | 3.76 | 3.64 | 3.91 | 3.91 |
| 4a-$CH_2$ | 3.71 | 3.59 | 3.61 | 4.0–2.7 | 3.71 | 3.73 (m) |
| 7d-$CH_3$ | 3.58 | 3.54 | 3.48 | 3.54 | 3.57 | |
| 1-$CH_3$ | 3.47 | 3.36 | 3.26 | 2.93 | 3.50 | 3.49 |
| 3-$CH_3$ | 3.27 | 3.14 | 3.20 | 2.43 | 3.29 | 3.30 |
| 7b-$CH_2$ | 2.51 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| 7a-$CH_2$ | 2.20 | 2.20 | 2.20 | 2.20 | 2.20 | 2.20 |
| 2a-$CH_3$ | 2.11 | | 1.62 | | 2.12 | 2.14 |
| 5b-$CH_3$ | 1.95 | 1.94 | 1.92 | 1.92 | 1.96 | 1.97 |
| 4b-$CH_3$ | 1.70 | 1.65 | 1.62 | 1.51 | 1.70 | 1.71 |
| 8-$CH_3$ | 1.46 | 1.45 | 1.42 | 1.51 | 1.50 | 1.50 |
| 2a-$OCH_3$ | | | | | 3.56, 3.53 | |
| 2a-$OCH_2CH_3$ | | | | | | 3.74 |
| 2a-$OCH_2CH_3$ | | | | | | 1.45, 1.32 |
| 7d-$OCH_2CH_3$ | | | | | | 4.05 |
| 7d-$OCH_2CH_3$ | | | | | | 1.12 |

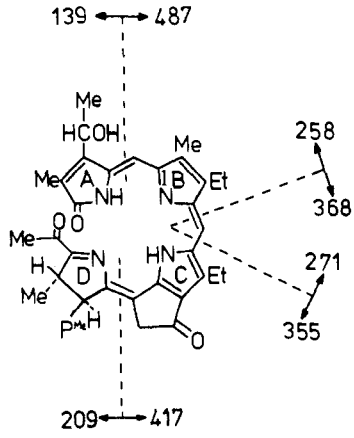
[a] Measured in $CDCl_3$ at 100 MHz. Shifts in δ.

The $^1H$ NMR spectrum of the derivative **8** was very interesting. Comparison with the methyl pheophorbides' spectrum showed that, instead of the expected one extra peak for the methyl of a methoxyl group, there are two peaks at δ 3.56 and 3.52. All the other resonances in the two spectra were virtually identical. A spectrum of the 2-methoxyethyl compound **8** was obtained using a 300-MHz instrument, and the expanded spectrum showed even more clearly the two peaks. Table V lists the $^1H$ NMR resonances of derivatives **6** and **8**, and Table I lists their $^{13}C$ resonances.

There are two plausible mechanisms which can account for the formation of derivative **8**. First, there could be direct displacement of hydroxy from the protonated 2-hydroxyethyl group by methoxy in an $S_N2$ manner. Alternatively, and more likely, loss of hydroxy could give a "benzylic-like" carbonium ion, which is then attacked by methanol. The carbonium ion might also lose a proton to give a vinyl group. It can only be assumed that the two peaks in the $^1H$ NMR spectrum correspond to different diastereoisomers, because the methoxyl compound is formed by a reaction at an asymmetric center, probably by way of a planar carbonium ion.[15]

Risch et al.[4] have also reported the preparation of the 2-methoxyethyl compound **8**; the $^1H$ NMR spectrum is the same as our own except that the methyl of the methoxyl group is assigned as a singlet at δ 3.68. The 2-methoxyethyl derivative **8** was therefore prepared following the same procedure used by Risch, i.e., by refluxing the methyl pheophorbides **6** in 4% sulfuric acid in methanol. A $^1H$ NMR spectrum of the resul-

**Scheme I.** Expected Mass Spectral Fragmentation Patterns for Photobilins **12**




tant derivative was identical with the spectrum of our material formed as a byproduct during the preparation of the methyl pheophorbides **6**; two peaks for the methyl of the methoxyl group were still present even at 100 MHz. There appears to be no logical explanation for the difference between the Braunschweig sample and our own.

It was thought that perhaps the two peaks in our sample were not due to diastereoisomeric methoxyl groups but due to the 1-methyl signal being split into a doublet because of its close proximity to the asymmetric center. The methyl of the methoxyl group could have been the singlet assigned to the 1-methyl group at $\delta$ 3.50. This theory was discounted by preparation of the 2-ethoxyethyl derivative **9** and examination of its $^1$H NMR spectrum. If the 1-methyl signal was a doublet, it would still be the same in the spectrum of the 2-ethoxyethyl compound **9**. This was shown not to be the case. The two peaks disappeared, being replaced by a multiplet at $\delta$ 3.74. It should be pointed out that the methyl ester was transesterified with ethanol during the preparation of **9** and so the singlet at $\delta$ 3.57 became a quartet at $\delta$ 4.05 and a triplet at $\delta$ 1.12. The multiplet at $\delta$ 3.74 (100 MHz) does not reveal whether two ethoxy compounds are present, but at higher field (220 MHz) examination of the triplet due to the methyl of the ethyl group shows that there are in fact again two ethoxyl group signals. At $\delta$ 1.34 there are clearly two superimposed triplets giving the appearance of a quartet. A minor mutiplet observed at $\delta$ 1.26 is presumed to be from resonance of terminal methyls of the 4-*n*-propyl and 4-isobutyl groups present in the minor homologues (see later for synthetic studies on this problem). Thus, it would seem that two diastereoisomeric ethoxy derivatives have also been formed.

### NMR Studies of Photobilins

One of the drawbacks in studying the bacteriochlorophylls *c* is their extreme susceptibility to breakdown. Apart from the demetalation, hydrolysis, and dehydration reactions discussed earlier, these pigments are extremely readily attacked by aerial oxygen.[2b] The oxygen adds across the $\delta$ carbon and the adjacent carbon on ring A, opening the macrocycle in an electrocyclic manner to form a homologous mixture of 8′-acetylbilitrienes **5**. We had earlier assumed[2b] that the oxidation was occurring during the growth of the bacteria, since this was not under completely anaerobic conditions. However, further work showed that the ring opening was not a result of the growth being incompletely anaerobic (while the chlorophylls are bound to cells photooxidation is probably unlikely). Oxidation was occurring during the preparation and workup of the methyl pheophorbides **6**.

The unique orientation of the ring opening was established by electron impact mass spectrometry. Examination of the mass spectrum of the photooxidation product of the pheophorbides from *C. ethylicum* showed mass ions for a series of homologues, *m/e* 612, 626, 640, and 654; the major peaks had *m/e* 626, which corresponded to structure **11** with 4-Et, 5-Et. The three main mass ions showed losses of 43 mass units ($CH_3CO$) with corresponding metastable ions. They also showed a loss of 87 mass units ($CH_2CH_2CO_2CH_3$) characteristic of a benzylic cleavage from a reduced pyrrole ring. Cleavage between the $\gamma$ and 7 positions, and between the $\alpha$ and 2 positions (Scheme I), would lead to fragments of *m/e* 209, 417 and 139, 487, respectively, from the proposed structure; peaks at *m/e* 419 and 487 are indeed observed. Furthermore, cleavage between the $\beta$ and 4 positions and between the $\beta$ and 5 positions would give fragments at *m/e* 368, 258, and 355, 271, respectively. Peaks at 368, 260, and 356 are observed; in addition, the two ions corresponding to the fragment half of rings C and D show losses of 43 mass units ($CH_3CO$). Thus, the mass spectrum confirms the proposed structure **11** (major component 4-Et, 5-Et), and gives no indication that the acetyl group resides at the 1′ rather than the 8′ position.

The methyl 2-vinylpheophorbides **2** and the methyl 2-methoxyethylpheophorbides **8** were shown to be equally prone to ring cleavage, and the corresponding derivatives **10** and **11** were both isolated and examined by mass and $^1$H NMR spectroscopy. Derivative **10** appeared to be one isomer, the mass spectrum showing a prominent peak at *m/e* 368. Derivative **11** was slightly more interesting. A mass spectrum showed the usual peak at *m/e* 368 and, as with derivatives **12** and **10**, no fragmentation of the "lower half" with ring D bearing a lactam group was observed. Again, it appeared as if only one mode of oxidation had taken place. The $^1$H NMR spectrum, however, suggested that both isomers have been formed, i.e., a mixture of compounds bearing an acetyl group on ring A and on ring D. The $\alpha$-methine proton at $\delta$ 6.34 was a doublet, indicating a mixture, and the signal at $\delta$ 2.23 for the acetyl group was slightly split into two. There seems to be no apparent reason why the ring opening should proceed differently when one considers that the other two derivatives **12** and **10** are pure isomers. The doublet methine proton signal and the split acetyl signal may be due to different helical forms of the tetrapyrrole, or the $\alpha$-methine proton signal may be split because of its close proximity to the asymmetric center, and the acetyl group may be lying under the asymmetric center in the helical form. The reason for the single methoxyl peak could be that the diastereoisomers mentioned previously are not chemically equivalent. There is always the possibility that the two peaks for the methoxyl group of compound **8** are due to a steric hindrance of rotation. If this is the case, relief of distortion of the ring when it is opened would allow easier movement, hence the single peak at $\delta$ 3.36.

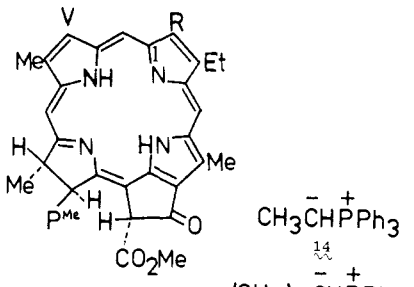
On this basis, if the $\alpha$-methine proton of derivative **11** is a doublet, then the $\alpha$ meso proton of compound **8** should also be a doublet. It is not, however, but close examination of the spectra does show a shoulder on the $\beta$ meso proton peak at $\delta$ 9.52. It can only be inferred that when the compound is ring opened any distortion of ring A caused by the $\delta$ methyl substituent is eliminated, thereby making the asymmetric center lie closer to the $\alpha$-methine proton. In fact, it might be expected[16] that the ease of electrophilic attack at the $\alpha$ meso position (e.g., deuterium exchange) is partly due to a distortion of the macrocycle by the $\delta$ meso substituent.

Finally, a noticeable feature of the $^1$H NMR spectra of all three photooxidation products, **12, 10,** and **11,** is that the methylene of the cyclopentanone shows as an AB quartet, whereas in the corresponding methyl pheophorbides it appears as a singlet. $^1$H NMR spectra of methyl mesopyropheophorbide *a* and *b* show an AB quartet, but the corresponding $\delta$ bromo compounds show a singlet.[13] This seems to indicate that a $\delta$ substituent does distort the macrocycle in such a way as to

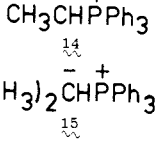
"push down" ring D, bringing the methylene in line with the propionate group and making the protons of the cyclopentanone ring, which are usually diastereotopic, equivalent, and thus a singlet in the NMR spectra.

### Partial Syntheses of *n*-Propyl- and Isobutylchlorins

On the basis of comparisons with benzenoid models, the carbon-13 resonances of terminal methyl groups of the *n*-propyl and isobutyl groups in bacteriochlorophyll *c* pheophorbides have been assigned to 14.4 and 23.1 ppm, respectively, and these values have been confirmed by $^{13}C$ and $^{13}CD_3$ methionine feeding experiments.[2b,5] However, the proton resonances from such substituents need clarification, so we embarked on a program to synthesize some appropriately substituted chlorins from available plant chlorophyll degradation products. In this way, definitive proton assignments for these groups might be secured. The key starting material was methyl pheophorbide *b* (**13**), readily available by degradation from chlorophyll *b*. Since alkene substituents at position 4 in the bacteriochlorophyll *c* precursors may be biosynthetically important,[2b] we chose to develop a route, using models, which would incorporate synthetic routes to such compounds. NMR assignments of alkene groups might also facilitate identification of such precursors isolated from bacterial cultures in the future. It was intended to construct the alkyl groups at the 3 position by first a Wittig reaction of either the ethyltriphenylphosphonium ylide **14** or the isopropyl ylide **15** with the 3-formyl group. Hydrogenation of the resulting alkene functions should produce the required alkyl groups, and simultaneously reduce the 2-vinyl group to an ethyl substituent. Obviously methyl pyropheophorbide *b* (**16**) could not be used as the starting compound because the isocyclic ring carbonyl would react with the phosphorus ylides. Methyl pheophorbide b (**13**) was therefore chosen. The methoxycarbonyl group on the isocyclic ring could be conveniently removed at the end of the syntheses, if necessary.



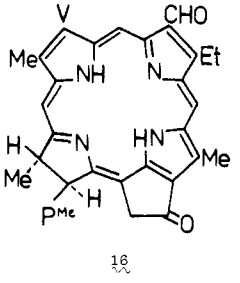
13 R = CHO
25 R = Me



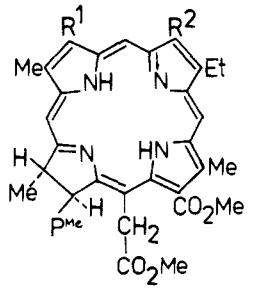
14



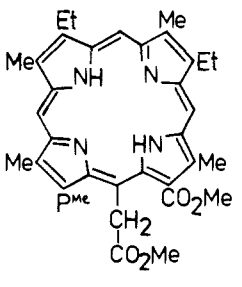
15



16



17 $R^1$ = CH=CH$_2$; $R^2$ = CHO
18 $R^1$ = CH=CH$_2$; $R^2$ = CH=CHCH$_3$
19 $R^1$ = Et; $R^2$ = n-Pr
20 $R^1$ = CH=CH$_2$; $R^2$ = CH=C(CH$_3$)$_2$
21 $R^1$ = Et; $R^2$ = i-Bu
23 $R^1$ = CH=CH$_2$; $R^2$ = Me



22

**3-*n*-Propyl Derivative.** Methyl pheophorbide *b* (**13**) was prepared from pheophytin *b*, isolated initially as the Girard-T complex, from a mixture of pheophytins *a* and *b*.[17] Ethyltriphenylphosphonium bromide was prepared in quantitative yield by reaction of ethyl bromide with triphenylphosphine in a sealed tube at 100 °C.[18] The required ylide **14** was generated in tetrahydrofuran using *n*-butyllithium, but reaction with methyl pheophorbide *b* proved unsuccessful. No product could be isolated, and the starting compound seemed to suffer decomposition or polymerization. The proton on the isocyclic ring was very easily removed, being part of a β-keto ester system, and it is well known that an anion at this position is extremely reactive, and aerial oxidation occurs readily.[17,19] It is probable that many side reactions were taking place and consuming the methyl pheophorbide *b*. To avoid this, the isocyclic ring was opened to form rhodin $g_7$ trimethyl ester (**17**), which hopefully would give the Wittig product more satisfactorily.

The opening of the cyclopentanone ring was originally reported by Fischer.[19] We improved the method using sodium methoxide instead of potassium hydroxide, giving rhodin $g_7$ trimethyl ester (**17**) directly, in very good yield, without the need for reesterification, and in this way rhodin $g_7$ trimethyl ester was prepared in 48% yield.

Reaction of **17** with the ethyltriphenylphosphonium ylide **14** gave the Wittig product, the 3-*n*-propenyl derivative **18,** in 23% yield after purification by column chromatography. The yield was low presumably because reactivity of the 3-formyl group is reduced because of electron donation from the electron-rich macrocycle. Also, there may be steric interference between the bulky triphenylphosphonium ylide and the large tetrapyrrolic unit, hindering the reaction.

Before hydrogenating the compound **18,** its zinc complex was prepared using zinc acetate in methanol. (Hydrogenation of a metal-free chlorin produces a form of porphyrinogen which decomposes as it reoxidizes.) The zinc complex of **18** was hydrogenated over palladized charcoal to the 3-*n*-propyl derivative **19,** zinc complex, the 2-vinyl group being reduced simultaneously. The reduction was not straightforward, however. Prolonged hydrogenation was necessary and, even then, larger scale reductions never went to completion. Removal of zinc was accomplished using trifluoroacetic acid to give **19**.

**3-Isobutyl Derivative.** The 3-isobutenyl derivative **20** was produced in 23% yield from reaction of rhodin $g_7$ trimethyl ester (**17**) with the isopropyltriphenylphosphonium ylide (**15**), prepared in the same way as the ethyl ylide **14** from isopropyltriphenylphosphonium iodide and *n*-butyllithium. The zinc complex of **20** was extremely resistant toward hydrogenation. Adams catalyst, as well as palladized charcoal, were tried, and different solvents, but all without success. Vast excesses of catalyst were also used. In every case a mixture of the zinc complexes of required compound **21** and the 3-isobutenyl derivative **20** was produced. Even when the hydrogenation was repeated several times, using fresh catalyst each time, complete reduction could not be achieved, probably due to steric effects. However, sufficient material was obtained to allow us to obtain the required data. No attempts were made to convert the materials into the corresponding mesopheophorbides, since it was not felt that significant changes in the chemical shifts would be apparent.

Bacteriochlorophyll *c* derivatives always show a multiplet at high field. This presumably consists of a triplet for the terminal methyl of a 4-*n*-propyl group and a doublet for the terminal methyls of a 4-isobutyl group. The 3-*n*-propyl model **19** does indeed show a triplet at δ 1.27 and the 3-isobutyl compound **21** a doublet at δ 1.25 for the terminal methyls of their respective groups. It is unfortunately difficult to assign the remaining alkyl protons because they are overshadowed in the spectra by other signals.

In anticipation of future partial syntheses,[8,20] we also de-

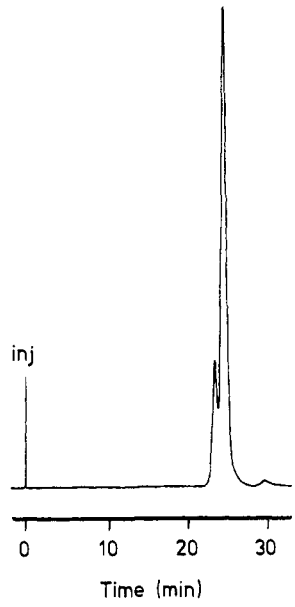



**Figure 2.** Partial separation of methyl pheophorbides **6** from *C. ethylicum* using normal-phase $\mu$-Porasil columns (2 3.9 mm i.d. × 30 cm). Conditions: 1 mL/min of $CHCl_3$, 1500 psi.

veloped a method for cyclization of the 6 and $\gamma$ substituents in chlorophyll derivatives to give the cyclopentanone ring. Fischer[19] used potassium hydroxide to produce a carbanion on the methylene of the meso methoxycarbonylmethyl group of rhodin $g_7$ trimethyl ester (**17**), which displaces methoxide from the adjacent nuclear methoxycarbonyl group in a Dieckmann-type cyclization. Low yields were usually obtained. Potassium *tert*-butoxide was found to be a more effective cyclization agent. The formation of the isocyclic ring was studied in porphyrins using chloroporphyrin $e_6$ trimethyl ester (**22**) and in chlorins using chlorin $e_6$ trimethyl ester (**23**). Chloroporphyrin $e_6$ trimethyl ester in refluxing pyridine containing potassium *tert*-butoxide was converted in 55% yield to pheoporphyrin $a_5$ dimethyl ester (**24**). Similarly, chlorin $e_6$ trimethyl ester (**23**) was converted to methyl pheophorbide *a* (**25**). Obviously, in the formation of the isocyclic ring, the two possible epimers are formed, but this is of no concern since it has been shown,[21] in studies on chlorophylls *a* and *a'* (the C-10 epimer), that equilibration by enolization of the cyclopentanone ring carbonyl group produces mainly the correct, thermodynamically most stable epimer.

## Separation of the Homologous Mixture

**Isolation of the Pigments from *C. ethylicum*.** The pure chlorophylls **1** are difficult to handle, since demetalation and hydrolysis of the farnesyl ester occur very easily. Also, the low oxidation potential of the magnesium complexes renders them very susceptible to photooxidation. Thus, all of our work on the chemistry and structures of these compounds has been performed on the methyl pheophorbide mixture, obtained by treatment of the extracted chlorophylls with sulfuric acid in methanol, which removes the magnesium and transesterifies the farnesyl ester. A 3.5-g supply of this material was built up by extracting large quantities of culture medium which had been allowed to grow for 2 weeks. The bacteria were found to be producing much more chlorophyll than earlier observed. We initially obtained yields of around 10 mg/L, but on extraction of 40 L of medium, over 1 g of the methyl pheophorbides was obtained, a yield of 25 mg/L. On column chromatography of the methyl pheophorbides, a faster moving chlorin band was obtained in varying amounts. This was a mixture of the 2-vinyl- and 2-methoxethylpheophorbides and could be minimized by using 3% v/v $H_2SO_4$ in methanol, and only leaving the compounds in methanol/sulfuric acid for 3-4 h instead of overnight, as had been the previous practice.

**Chromatographic Separations.** Holt[22] originally separated the pheophorbides by distribution between HCl and ether on Celite columns. This method did not seem applicable to our needs, as in planned radioactive feeding work only small amounts of material are being handled. Holt only obtained 47% recovery in his separations, clearly not good enough for our purposes. MacDonald[23] has determined that many of Holt's fractions are contaminated with varying amounts of neighboring bands.

Using various systems, we attempted separation by preparative silica TLC, without any success, even when using continuous elution. The ease of degradation of these compounds would also rule this method out had even a partial separation been achieved. Richards and Rapoport[24] achieved a partial resolution using countercurrent distribution. They also reported[25] a preparation of pure band 5 by repeated chromatography on powdered polythene columns. Again these methods did not seem suitable for our feeding experiments.

The obviously desirable choice would be high-performance liquid chromatography (HPLC). This relatively recent technique employs pressurized solvent flow, coupled with extremely fine column packing, and is capable of achieving high resolution of complex mixtures in short periods of time. An added advantage is that as the system is completely enclosed, there is no chance of the compounds suffering photooxidative degradation during the separation. The technique is particularly suitable for separation of milligram quantities of material. Porphyrin-type isomers have been resolved by HPLC,[26] and it certainly appeared the most promising technique.

Using two normal phase analytical columns in series, and eluting with chloroform (containing 0.75% ethanol), the chromatogram in Figure 2 was obtained, showing a definite separation of one (or more) of the minor bands. The smaller peak could be obtained pure (on a small scale) by multiple recycling and clipping the major band. The visible spectrum showed that it was a *Chlorobium* pheophorbide, thus discounting the possibility that it was some sort of degradation product. In fact, the 2-methoxyethyl pheophorbides **8** and the photooxidation product **12** were well separated by this system. This separation was encouraging, but complete resolution of the mixture did not appear promising by normal phase chromatography.

Encouraged by Strouse's report[27] of efficient separation of the chlorophylls from *Chlorobium limicola* using reverse phase chromatography on powdered polyethylene, we decided to attempt the separation by reverse phase HPLC. Using one C-18 $\mu$-Bondapak column (Waters Associates) and 33% water in acetonitrile as the solvent, the chromatogram in Figure 3 was obtained, indicating a definite separation of the three bands. It is clearly visible that there are four major bands; mass spectrometry identified fractions F1 with Holt's band 5 and F3 with band 2. By recycling the two center bands, these were resolved (see inset, Figure 3). Overloading the column and clipping the ends of the bands enabled small amounts of F2a and F2b to be obtained pure.

The mass spectra were almost identical (Figure 4) and showed that they had the same molecular weight, 608. Since the major part of this fraction has the structure 4-*n*-Pr, 5-Et, then one possibility for the minor band F2b is 4-*i*-Bu, 5-Me. This is probably unlikely for, if there is no component with 4-Et, 5-Me substituents (i.e., Holt's fraction 6), it is unlikely biosynthetically that any higher homologue containing a 5-Me group would be present.

There are several other possibilities for the structure of F2b. From the chromatographic behavior and molecular weight, it has to have one extra methyl group compared to fraction F1 (4-Et, 5-Et). Three plausible structures are **26**, **27**, and **28**. The

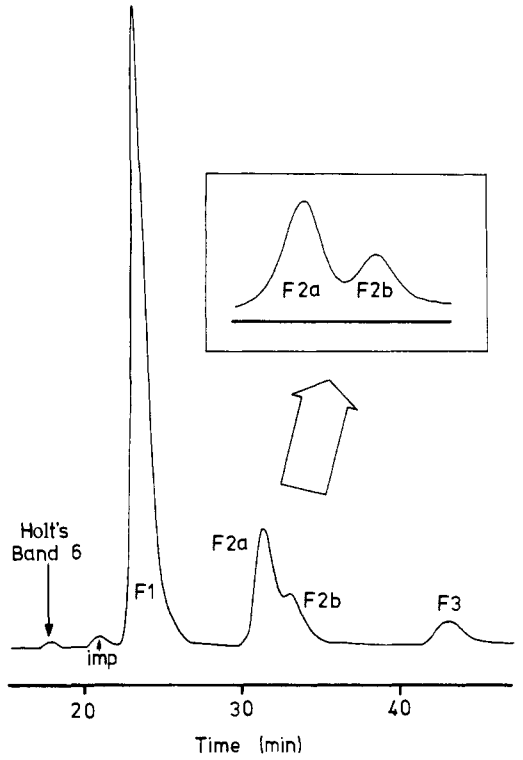
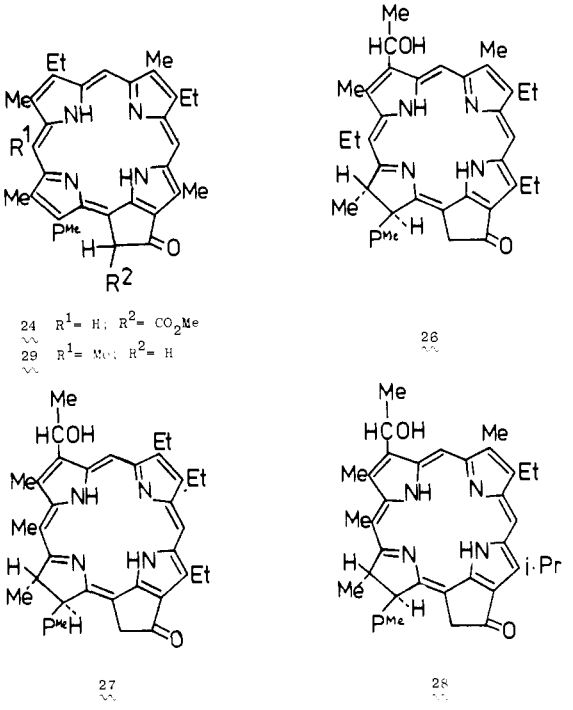



**Figure 3.** Separation of methyl pheophorbides **6** from *C. ethylicum* using reverse-phase $\mu$-Bondapak C-18 column (7.8 mm i.d. × 30 cm). Conditions: 2 mL/min of 33% $H_2O/CH_3CN$, 2000 psi. The inset shows the F2a/F2b portion of the chromatogram after one recycle. A similar picture was obtained without recycle if two $\mu$-Bondapak 7.8 mm i.d. × 30 cm columns were used in series. One further recycle of the inset separation (one column) gave an excellent base-line separation of F2a and F2b.

meso ethyl structure **26** is unlikely biosynthetically. The structure **27** with a 3-ethyl group could arise from methylation




of the acetic acid side chain at the 3 position before decarboxylation. A 5-*i*-Pr side chain might be considered likely if the mechanisms of methylation are as hypothesized.[2,5] Two methylations of the 5-acetic acid side chain followed by decarboxylation would give the isopropyl side chain. Brockmann's degradation work would have been expected to reveal the

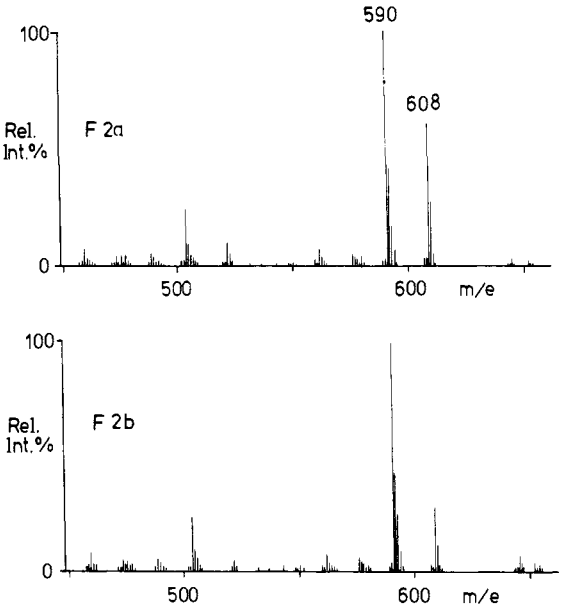



**Figure 4.** Electron impact mass spectra of methyl pheophorbides from fraction F2a (upper) and fraction F2b (lower).

presence of a 5-*i*-Pr group if present.[3] Attempts to scale up the separation to further identify the structure of F2b are in hand. When acetone/water mixtures were employed as the solvent system, the separation of fraction 2 into the separate bands was less marked.

The percent composition of the mixture was estimated by integration of the bands on the chromatogram. The estimated composition is shown in Table VI. Band 6 makes up only 0.2% of the total mixture. When our biosynthetic investigations were begun, the organism grown was *Chlorobium thiosulfatophilum*. A small amount of the pheophorbides from these studies was available, and HPLC analysis of the mixture again indicated that Holt's band 6 was a minor component (2.2%). The estimated composition is also shown in Table VI.

**Separation Work on Other Degradation Products.** To show that none of the bands obtained earlier were merely degradation products, HPLC separation was attempted on some of the common degradation products. The methyl mesopheophorbides showed three bands only; there was no sign of a separation of band 2 into two components, even on multiple recycle. A sample[8] of partially synthetic band 6, **29**, was added to the natural mixture, and the two chromatograms are shown in Figure 5. This identifies one of the minor peaks as being derived from Holt's band 6; the others are possibly decomposition products, as the sample had been stored for several years. The 2-vinylpheophorbides **2** likewise showed no tendency for the second fraction to split into two, despite variation in the solvent system and multiple recycling. The 2-methoxyethylpheophorbides **8** isolated from a large scale extraction of the methyl pheophorbides initially appeared to consist of four bands, but this was due to the presence of some 2-vinylpheophorbide impurity.

The much improved separation on reverse-phase columns compared with normal phase can be understood because the polarity of the individual homologues is not significantly different, since they contain the same functional groups. In reverse-phase chromatography, compounds which are the most polar, and hence make weaker interactions with the very nonpolar support, are eluted first. Thus, in reverse phase, compounds which have differences in the nonpolar parts of the molecule are more easily separated, with the most highly methylated homologues (in our case) being retained longer on the columns. It has already been mentioned that preparative

**Table VI.** Estimated Composition of Pheophorbides in Chromatographic Bands

| fraction | Holt band no. | $R^1$ (4 substit) | $R^2$ (5 substit) | % age of mixture[a] | mol wt |
|---|---|---|---|---|---|
| | | A. Methyl Pheophorbides from *Chloropseudomonas ethylicum* | | | |
| | 6 | Et | Me | 0.2 | 580 |
| F1 | 5 | Et | Et | 71.7 | 594 |
| F2a | 4 | *n*-Pr | Et | 18.3 | 608 |
| F2b | | ? | ? | 5.3 | 608 |
| F3 | 2 | *i*-Bu | Et | 4.5 | 622 |
| | | B. Methyl Pheophorbides from *Chlorobium thiosulfatophilum* | | | |
| | 6 | Et | Me | 2.2 | 580 |
| F1 | 5 | Et | Et | 65.8 | 594 |
| F2a | 4 | *n*-Pr | Et | 25.8 | 608 |
| F2b | | ? | ? | 4.2 | 608 |
| F3 | 2 | *i*-Bu | Et | 2.0 | 622 |

[a] Estimates of percentage composition assume equal extinction coefficients for all bands at 405 nm.

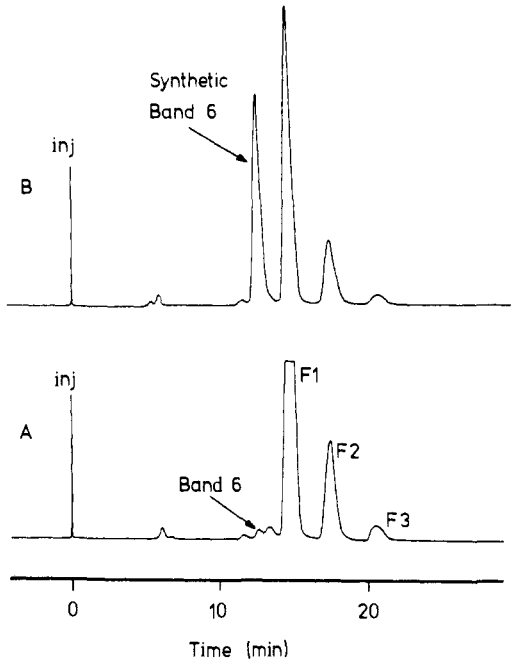



**Figure 5.** Reverse-phase HPLC separations of methyl mesopheophorbides **7** from *C. ethylicum* using a μ-Bondapak C-18 column (7.8 mm i.d. × 30 cm). Conditions: 2 mL/min of 10% $H_2O/CH_3CN$, 2000 psi. (A) Methyl mesopheophorbides obtained by dehydration and reduction of the natural pheophorbide mixture, **6**. (B) As in A, but sample has been doped with synthetic band 6 methyl mesopheophorbide[8] (related to **1f**).

TLC on silica gel failed to achieve any separation. An analytical TLC was run on cellulose, a polar support, eluting with aqueous methanol. The front and rear of the band were scraped off, and the pigments analyzed by LC. This showed that there was a substantial difference in the composition of the two portions taken. At the front of the band the ratio of the fractions 1, 2, and 3 was approximately 40:6:1. The rear portion of the band contained substantially less of the first fraction, the ratio of homologues being about 5.2:4.8:1. Large scale separation of the homologues by HPLC might in the future be facilitated by prior preparative TLC on cellulose plates.

## Experimental Section

**General Conditions.** Melting points were measured on a hot-stage apparatus, and are uncorrected. HPLC was performed using a Waters Associates instrument consisting of a Model 6000A solvent delivery system, U6K injector, and Model 440 absorbance detector, set at 405 nm, connected to an external strip recorder. Normal phase columns (30 cm × 3.9 mm i.d.) were packed with μ-Porasil; reverse-phase columns (30 cm × 7.8 mm i.d.) were packed with C-18 μ-Bondapak. The acetone and acetonitrile used were distilled from $K_2CO_3$ and aqueous solvents were degassed by heating to a slow boil while stirring. Neutral alumina (Merck), usually Brockmann Grade III (6% $H_2O$) or Grade V (15% $H_2O$), was used for column chromatography. Preparative TLC was carried out on glass plates coated with Merck GF 254 silica gel (1.5 mm thickness), and analytical TLC was carried out using Merck silica gel 60 F-254 precoated sheets (0.2 mm thickness). Electronic absorption spectra were carried out with a Unicam SP-800 or Cary 17 spectrophotometer using solutions in methylene chloride. Proton NMR spectra were recorded at 100 MHz (unless stated otherwise) on a Varian XL-100 using solutions in deuteriochloroform with tetramethylsilane as added internal standard. High-field NMR spectra were run on Perkin-Elmer PE-34 (220 MHz), Varian SC-300 (300 MHz), or Nicolet NT-360 (360 MHz) spectrometers. Full details of the conditions for $^{13}C$ spectra (Varian XL-100) have been given elsewhere.[12] Mass spectra were measured usually on MS 902 or MS 12 spectrometers (direct insertion probe, 70 eV, 50 μA, source temperature ca. 200 °C). Conditions for the growth of *C. ethylicum* have been described elsewhere.[5]

**Ethyltriphenylphosphonium Bromide (14).** Ethyl bromide (5 g) and 11 g of triphenylphosphine were heated in a sealed tube at 100 °C during 1 h. The resulting white solid was washed with 2 × 100 mL of benzene and 2 × 100 mL of ether and then dried, to give the phosphonium salt (15.5 g, 91%) as white plates: mp 203–208 °C; NMR δ 7.80 (15 H, m, 3 × Ph), 3.90 and 3.76 (2 H, 2 × q, $CH_2$), 1.50 and 1.30 (3 H, 2 × t, $CH_3$).

**Isopropyltriphenylphosphonium Iodide (15).** Isopropyl iodide (5.5 g) and 10.0 g of triphenylphosphine were heated in a sealed tube at 100 °C during 5 h. As in the foregoing preparation, the white solid was washed thoroughly with benzene and ether and dried to give white plates (7.5 g, 45%): mp 194–196 °C (lit.[18] 191 °C); NMR δ 7.90 (15 H, m, 3 × Ph), 5.12 (1 H, m, C*H*), 1.46 and 1.26 (6 H, 2 × d, 2 × $CH_3$).

**Rhodin $g_7$ Trimethyl Ester (17).** A solution of 2 g of methyl pheophorbide *a*[17] in 80 mL of dry tetrahydrofuran was stirred during 1 h at room temperature under nitrogen with a solution of 400 mg of sodium in 80 mL of dry methanol. Glacial acetic acid (1.2 mL) was added and the solution was shaken with 200 mL of methylene chloride and 200 mL of water. The organic phase was washed with 100 mL of water, dried over $Na_2SO_4$, and evaporated to a residue which was first treated with excess ethereal diazomethane and then chromatographed on Grade V alumina, eluting with 20% methylene chloride in toluene. Evaporation of the dark red-brown eluates gave a blue-black solid. Recrystallization from methylene chloride–light petroleum (bp 60–80 °C) gave black needles (1 g, 48%): mp 241–243 °C (lit.[19] 251 °C); NMR δ 11.1 (1 H, s, C*H*O), 10.25, 9.65, and 8.65 (each 1 H, s, 3 × meso *H*), 8.0 (1 H) and 6.28 (2 H) (both m, C*H*=C$H_2$), 5.27 (2 H, q, meso C$H_2$), 4.40 (2 H, m, 7- and 8-*H*), 4.28, 3.81, 3.68, 3.52, and 3.40 (each 3 H, s, 5 × C$H_3$), 4.05 (2 H, q, C$H_2$CH$_3$), 2.60 and 2.30 (both 2 H, m, C$H_2$C$H_2$), 1.80 (3 H, t, CH$_2$C$H_3$), and 1.78 (3 H, d, 8-C$H_3$); vis $\lambda_{max}$ 408 (ε 61 700), 429 (173 500), 524 (10 900), 556 (7700), 592 (6000), and 650 nm (23 000); MS *m/e* 652 ($M^+$, 100%). Anal. Calcd for $C_{37}H_{40}N_4O_7$: C, 68.08; H, 6.18; N, 8.58. Found: C, 68.36; H, 6.26; N, 8.81.

**3-(1-Propenyl)-3-deformylrhodin $g_7$ Trimethyl Ester (18).** Ethyltriphenylphosphonium bromide (1.37 g, 3 equiv) was stirred at 0 °C with 0.24 g (3 equiv) of *n*-butyllithium (added as a 2 M solution in 1.9

mL of hexane) in 80 mL of tetrahydrofuran under nitrogen during 20 min. To this was added 800 mg of rhodin $g_7$ trimethyl ester (**17**) and the mixture was stirred overnight at room temperature. Methylene chloride (600 mL) was added and the solution was washed with 3 × 200 mL of water, dried over $Na_2SO_4$, and evaporated to dryness. After we treated the residue with excess ethereal diazomethane, it was chromatographed on Grade V alumina eluting with 50% methylene chloride in toluene. Evaporation of the dark green eluates gave a black solid. Recrystallization from methylene chloride–light petroleum ether (bp 60–80 °C) gave the 3-*n*-propenyl derivative (190 mg, 23%) as black microprisms: mp 126–129 °C; NMR δ 9.69, 9.64, and 8.69 (each 1 H, s, 3 × meso *H*), 8.02 (1 H) and 6.09 (2 H) (m, C*H*=C*H*$_2$), 7.57 (1 H) and 6.56 (1 H) (m, C*H*=C*H*), 5.27 (2 H, q, meso C*H*$_2$), 4.39 (2 H, m, 7- and 8-*H*), 4.25, 3.75, 3.62, 3.57, and 3.44 (each 3 H, s, 5 × C*H*$_3$), 3.70 (2 H, q, 4-C*H*$_2$CH$_3$), 2.4–2.1 (4 H, m, C*H*$_2$C*H*$_2$), 2.32 (3 H, d, =CHC*H*$_3$), and 1.77 [6 H, 8-C*H*$_3$ (d), 4-CH$_2$C*H*$_3$ (t)]; vis $\lambda_{max}$ 409 (ε 148 400), 504 (12 400), 534 (3200), 558 (2000), 606 (4360), and 662 nm (40 900); MS *m/e* 664 ($M^+$, 100%). Anal. Calcd for $C_{39}H_{44}N_4O_6$: C, 70.46; H, 6.67; N, 8.43. Found: C, 70.23; H, 6.89; N, 8.25.

**3-(1-Isobutenyl)-3-deformylrhodin $g_7$ Trimethyl Ester (20).** Isopropyltriphenylphosphonium iodide (1.33 g, 5 equiv) was stirred in 60 mL of tetrahydrofuran at 0 °C with 208 mg (5 equiv) of *n*-butyllithium (added in 1.6 mL of *n*-hexane) during 20 min under nitrogen. To this was added 400 mg of rhodin $g_7$ trimethyl ester (**17**), and the mixture was left overnight at room temperature. The reaction was worked up exactly as the foregoing preparation. Recrystallization from methylene chloride–light petroleum ether (bp 60–80 °C) gave the product (95 mg, 23%) as grey-black microneedles: mp 173–174.5 °C; NMR δ 9.72, 9.48, and 8.72 (each 1 H, s, 3 × meso *H*), 8.02 (1 H) and 6.22 (2 H) (both m, C*H*=C*H*$_2$), 7.16 (1 H, s, C*H*=), 5.29 (2 H, q, meso C*H*$_2$), 4.44 (2 H, m, 7- and 8-*H*), 4.27, 3.77, 3.64, 3.58, and 3.46 (each 3 H, s, 5 × C*H*$_3$), 3.75 (2 H, q, C*H*$_2$CH$_3$), 2.55 and 2.20 (both 2 H, m, C*H*$_2$C*H*$_2$), 2.82 and 1.86 [both 3 H, s, CH=C (C*H*$_3$)$_2$], and 1.75 [6 H, 8-C*H*$_3$ (d) and 4-CH$_2$C*H*$_3$ (t)]; vis $\lambda_{max}$ 407 (ε 151 000), 503 (14 100), 532 (4300), 557 (2500), 606 (5700), and 662 nm (38 600); MS *m/e* 678 ($M^+$, 100%). Anal. Calcd for $C_{40}H_{46}N_4O_6$: C, 70.77; H, 6.83; N, 8.25. Found: C, 70.58; H, 7.04; N, 8.44.

**3-*n*-Propyl-3-deformylmesorhodin $g_7$ Trimethyl Ester (19).** The 3-*n*-propenyl derivative **18** (100 mg) was warmed in 5 mL of methylene chloride with a solution of 100 mg of zinc acetate in 5 mL of methanol during 10 min. The solution was poured into 50 mL of water and extracted with 2 × 50 mL of methylene chloride. The organic phase was dried over $Na_2SO_4$ and evaporated to give the zinc complex of the *n*-propenyl derivative, which was immediately taken up in 40 mL of tetrahydrofuran containing 10 mg of 10% palladized charcoal and hydrogenated at room temperature and atmospheric pressure during 24 h. Filtration through Celite and evaporation gave a residue which was stirred in 5 mL of trifluoroacetic acid during 5 min. Water (50 mL) and methylene chloride were added, and the organic phase was washed with 50 mL of sodium bicarbonate solution, 50 mL of water, and dried over $Na_2SO_4$. Evaporation to dryness gave a residue which was chromatographed on Grade V alumina, eluting with 50% methylene chloride in toluene. The green eluates were evaporated to a green solid. Recrystallization from cold light petroleum ether (bp 60–80 °C) gave the *n*-propyl derivative (76 mg, 76%) as green microneedles: mp 156–158 °C; NMR δ 9.69, 9.36, and 8.65 (each 1 H, s, 3 × meso H), 5.28 (2 H, q, meso C*H*$_2$), 4.40 (2 H, m, 7- and 8-*H*), 4.26, 3.77, 3.62, 3.58, and 3.34 (each 3 H, s, 5 × C*H*$_3$), 3.72 [6 H, 2- and 4-C*H*$_2$CH$_3$ (q) and 3-CH$_2$ (t)], 2.5 and 2.3 (4 H, m, C*H*$_2$C*H*$_2$), 2.16 (2 H, m, CH$_2$C*H*$_2$CH$_3$), 1.75 [9 H, 8-C*H*$_3$ (d), 2- and 4-CH$_2$C*H*$_3$ (t)], and 1.27 (3 H, t, CH$_2$CH$_2$C*H*$_3$); vis $\lambda_{max}$ 398 (ε 146 000), 497 (12 000), 523 (3000), 548 (2000), 595 (5000), and 649 nm (37 000); MS *m/e* 668 ($M^+$, 100%). Anal. Calcd for $C_{39}H_{48}N_4O_6$: C, 70.03; H, 7.23; N, 8.38. Found: C, 69.90; H, 7.14; N, 8.47.

**3-Isobutyl-3-deformylmesorhodin $g_7$ Trimethyl Ester (21).** The 3-isobutenyl derivative **20** (90 mg) was converted to the zinc complex, hydrogenated, and then demetalated exactly as in the foregoing preparation. Recrystallization from methylene chloride–light petroleum ether (bp 60–80 °C) gave black prisms (50 mg, 56%): NMR δ 9.56, 9.32, and 8.49 (each 1 H, s, 3 × meso *H*), 5.12 (2 H, q, meso C*H*$_2$), 4.80 (2 H, m, 7- and 8-*H*), 4.20, 3.83, 3.67, 3.46, and 3.25 (each 3 H, s, 5 × C*H*$_3$), 3.8 and 3.4 (6 H, m, 2- and 4-C*H*$_2$CH$_3$ and 3-C*H*$_2$), 2.5 and 2.1 (4 H, m, C*H*$_2$CH$_2$), 1.75 (9 H, m, 8-CH$_3$, 2- and 4-C*H*$_3$), and 1.25 [(6 H, d, CH(C*H*$_3$)$_2$]. In addition, resonances at δ 7.16 for the isobutenyl single proton and at 2.36 and 1.86 for the terminal methyls of the isobutylidene group clearly showed that the required derivative contained about 10% of the 3-isobutenyl derivative **20** of mesorhodin $g_7$ trimethyl ester. A mass spectrum also indicated two components at *m/e* 682 and 680. In other experiments, hydrogenations were performed in acetic acid, methanol, and with Adams catalyst. Incomplete hydrogenation was observed every time.

**Methyl Pheophorbide *a* (25).** Chlorin $e_6$ trimethyl ester (**23**) (25 mg) was refluxed in 6 mL of pyridine containing 22 mg (5 equiv) of potassium *tert*-butoxide (added in 1.0 mL of *tert*-butyl alcohol) during 30 s. Glacial acetic acid (0.5 mL) was added, and the solution was poured into 20 mL of water and extracted with 3 × 20 mL of methylene chloride. The combined organic extracts were washed with 20 mL of water, dried over $Na_2SO_4$, and evaporated to dryness, azeotroping the pyridine with toluene. After treatment with diazomethane, the product was chromatographed on Grade V alumina, eluting with methylene chloride. Evaporation of the dark eluates and recrystallization from methylene chloride–methanol gave the product (16 mg, 67%) as blue plates: mp 222–224 °C (lit.[17] 224–226 °C; lit.[19] 228 °C).

**Pheoporphyrin $a_5$ Dimethyl Ester (24).** Chloroporphyrin $e_6$ trimethyl ester (**22**) (40 mg) was refluxed in 6 mL of pyridine containing 40 mg (5 equiv) of potassium *tert*-butoxide (added in 1 mL of *tert*-butyl alcohol) during 10 min. The reaction was worked up as in the foregoing preparation and gave the product (21 mg, 55%) as purple plates from methylene chloride–methanol: mp 273–275 °C (lit.[19] 273 °C). A small amount (3 mg) of starting material **22** was recovered and was identified by TLC comparison and NMR spectroscopy.

**Methyl 2-Devinyl-2-(1′-hydroxyethyl)-5-demethyl-5-ethyl-δ-methylpyropheophorbide *a* and Homologues, Methyl Pheophorbides 6.** A 40-L culture of *C. ethylicum*, grown for about 10 days from subculturing, was filtered through a 1-in. wet bed of Celite that was made by filtering a suspension of Celite in water (3:1 mixture of Celites 545 and 505). The Celite and pigments were stirred in 1.5 L of acetone during 2 h, and the resultant suspension was filtered to give a dark green solution. Methylene chloride (1 L) and 1 L of water were added, and the organic layer was washed with 500 mL of water and 500 mL of sodium chloride solution, and dried over $Na_2SO_4$. Evaporation to dryness gave a black oily residue which was stirred in the dark overnight in 500 mL of 5% sulfuric acid in methanol. The solution was poured into 200 mL of water and extracted with 2 × 200 mL of methylene chloride. The combined organic extracts were washed with 200 mL of water and 200 mL of sodium bicarbonate solution, and dried over $Na_2SO_4$. After evaporation to dryness, the black residue was chromatographed on Grade III alumina, eluting with toluene containing increasing amounts of methylene chloride. The black eluates were collected and evaporated to a blue-black glass (yield 1 g). For the $^1H$ NMR spectrum and assignments, see Table V; see Table I for the $^{13}C$ NMR spectrum and assignments: vis $\lambda_{max}$ I 669, II 614, III 557, IV 525, V 492, VI 424; VI > I > III > IV > II > V; MS *m/e* 622 ($M^+$, 7%), 608 ($M^+$, 24), 594 ($M^+$, 100) and 580 ($M^+$, 12). A less polar compound was sometimes isolated during chromatography. It was assigned the structure: methyl 2-devinyl-2-(1′-methoxyethyl)-5-demethyl-5-ethyl-δ-methylpyropheophorbide *a* and homologues, methyl 2-methoxyethylpheophorbides **8**. This compound was usually contaminated with the forerunning carotenoid fraction. Preparative TLC using six (20 × 20 cm) plates coated with silica gel and eluted with 3% tetrahydrofuran in methylene chloride gave, after extraction from the silica with 5% methanol in methylene chloride, filtration, and evaporation, a blue-black glass; yield usually about 100 mg. Full $^1H$ and $^{13}C$ NMR data can be found in Tables I and V: vis $\lambda_{max}$ I 670, II 614, III 550, IV 518, V 586, VI 414; VI > I > III > IV > II > V; MS *m/e* 636 ($M^+$, 5%), 622 ($M^+$, 23), 608 ($M^+$, 100), 594 ($M^+$, 9).

**Methyl 2-Devinyl-2-(1′-methoxyethyl)-5-demethyl-5-ethyl-δ-methylpyropheophorbide *a* and Homologues, Methyl 2-Methoxyethylpheophorbides 8.** Methyl pheophorbides **6** (40 mg) were refluxed in 100 mL of 4% sulfuric acid in methanol during 2.5 h. The solution was cooled and poured into 100 mL of methylene chloride and 100 mL of water. The organic phase was washed with 50 mL of water, 50 mL of sodium bicarbonate solution, and 50 mL of water, dried over $Na_2SO_4$, and evaporated to give a black residue. Chromatography on two (20 × 20 cm) preparative TLC plates coated with silica gel and eluted with 3% tetrahydrofuran in methylene chloride gave a black component which was extracted from the silica by stirring in 5% methanol in methylene chloride. The 2-methoxethyl derivative (30

mg, 73%) was obtained as a blue-black glass. It was identical by TLC, MS, and $^1$H NMR with the minor compound isolated in the foregoing large-scale experiment. In an analogous experiment, the 2-ethoxyethylpheophorbides **9** were prepared using 40 mg of methyl pheophorbides 6 dissolved in 100 mL of 4% sulfuric acid in ethanol: yield (30 mg, 71%); MS *m/e* 622 ($M^+$, 4%), 636 ($M^+$, 100), 650 ($M^+$, 29), 664 ($M^+$, 7). For the $^1$H NMR spectrum and assignment, see Table V.

**1,3,8-Trimethyl-4,5-diethyl-2-(1-hydroxyethyl)-6-γ-(1-oxoethylene)-7,8-dihydro-7-(methoxycarbonylethyl)-8′-acetylbilitriene and Homologues, Normal Photobilin (12).** A solution of methyl pheophorbides **6** from *C. ethylicum* in 200 mL of methylene chloride was purged in normal room lighting with air for 1 week. The solution was evaporated to dryness and the residue chromatographed using four (20 × 20 cm) preparative TLC plates eluted with 5% tetrahydrofuran in methylene chloride. The compound was isolated as in the foregoing preparation. A purple glassy solid (60 mg, 71%) was obtained: NMR δ 9.55 (br, N*H*), 6.89 and 6.46 (each 1 H, s, 2 × methine-*H*), 5.10 (1 H, q, C*H*OH), 3.70 (3 H, s, OC$H_3$), 3.58 (2 H, q, C$H_2$CO), 3.1–2.4 (m, 7- and 8-*H*, C$H_2$C$H_2$, 4- and 5-C$H_2$), 2.20 (3 H, s, C$H_3$CO), 2.10 and 1.89 (both 3 H, s, 2 × C$H_3$), 1.62 (3 H, d, CH(OH)C$H_3$), 1.38 (3 H, t, C$H_3$), and 1.20 [6 H, C$H_3$ (t), 8-C$H_3$ (d)]; vis $\lambda_{max}$ I 590, II 560, III 366, IV 320; VI > III > II > I; MS *m/e* 654 ($M^+$, 7%), 640 ($M^+$, 28), 626 ($M^+$, 100), 612 ($M^+$, 14), 597 (36), 583 (79), 368 (20), 325 (32), m* 557, 543.

**1,3,8-Trimethyl-4,5-diethyl-2-vinyl-6-γ-(1-oxoethylene)-7,8-dihydro-7-(2-methoxycarbonylethyl)-8′-acetylbilitriene and Homologues, Vinyl Photobilin (10).** Methyl 2-vinylpheophorbides **2** (100 mg) in 300 mL of methylene chloride were purged in normal room lighting with air for 1 week. The product, a purple glass (53 mg, 50%), was isolated as in the foregoing preparation: NMR δ 9.52 (br, N*H*), 6.90 and 6.14 (both 1 *H*, s, 2 × methine-*H*), 6.65 (1 H) and 5.72 (2 H) (m, C*H*=C$H_2$), 3.70 (3 H, s, OC$H_3$), 3.58 (2 H, q, C$H_2$CO), 3.1–2.4 (m, 7- and 8-*H*, C$H_2$C$H_2$, 4- and 5-C$H_3$), 2.17 (3 H, s, C$H_3$CO), 2.10 and 1.96 (both 3 H, s, C$H_3$), 1.40 (3 H, t, C$H_2$C$H_3$), and 1.20 [6 H, m, C$H_3$ (t) and 8-C$H_3$ (d)]; vis $\lambda_{max}$ I 586, II 558, III 360, IV 312; VI > III > II = I; MS *m/e* 636 ($M^+$, 6%), 622 ($M^+$, 30), 608 ($M^+$, 100), 594 ($M^+$, 10), 579 (30), 565 (80).

**1,3,8-Trimethyl-4,5-diethyl-2-(1-methoxyethyl)-6-γ-(1-oxoethylene)-7,8-dihydro-7-(2-methoxycarbonylethyl)-8′-acetylbilitriene and Homologues, 2-Methoxyethyl Photobilin (11).** Methyl 2-methoxyethylpheophorbides **8** (100 mg) in 500 mL of methylene chloride were purged with air in normal room lighting during 1 week. The product was isolated as in the foregoing preparations. A purple solid (70 mg, 67%) was obtained: NMR δ 9.60 (br, N*H*), 6.88 (1 H, s, methine-*H*), 6.34 (1 H, d, methine-*H*), 4.52 (1 H, m, C*H*OCH$_3$), 3.70 (3 H, s, $CO_2CH_3$), 3.58 (2 H, q, C$H_2$CO), 3.36 (3 H, s, CHOC$H_3$), 2.9–2.3 (m, 7- and 8-*H*, C$H_2$C$H_2$, 4- and 5-C$H_2$), 2.23 (3 H, d, C$H_3$CO), 2.10 and 1.88 (both 3 H, s, C$H_3$), 1.55 (3 H, d, C$H_3$CHOCH$_3$), 1.39 (3 H, t, C$H_3$), and 1.26 [6 H, m, C$H_3$ (t) and 8-C$H_3$ (d)]; vis $\lambda_{max}$ I 656, II 586, III 560, IV 410, V 368, VI 320; VI > V > IV > III = II > I; MS *m/e* 668 ($M^+$, 6%), 654 ($M^+$, 19), 640 ($M^+$, 87).

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