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Chemical Studies on Products Obtained from Verdohemochrome IXα Dimethyl Ester by Treatment with Ammonia under Air

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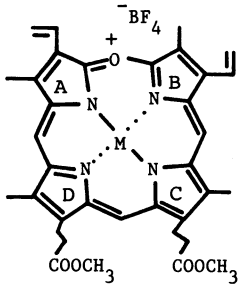
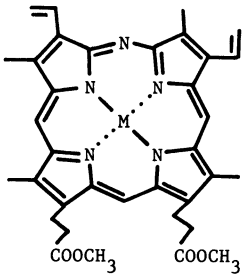
Verdohemochrome IXα dimethyl ester [bis(pyridine)iron(II) 5-oxaprotoporphyrin IX dimethyl ester] gave 5-azaprotohemin IX dimethyl ester (**2**) and six tripyrrin derivatives upon treatment with ammonia under air, followed by methylation. The reaction of **2** with concd HCl in acetic acid in the presence of ferrous sulfate, followed by methylation gave demetallated product, 5-azaprotoporphyrin IX dimethyl ester (**8**), accompanied by products obtained by the hydration and reduction of both or one of the vinyl groups of **8**. 8,12-Bis[2-(methoxycarbonyl)ethyl]-3,7,13-trimethyl-1-oxo-2-vinyltripyrrin-14-carbaldehyde, obtained as a major tripyrrin product by an oxidative degradation of **1**, gave a pyrromethene derivative, 3,7-bis[2-(methoxycarbonyl)ethyl]-2,8-dimethyl-1-methoxy-5-pyrromethene-9-carbaldehyde by the reaction with $Tl(OAc)_3$ under air. The structures of all products obtained in the present study were determined by spectral measurements.

In human beings and many animals, endogenous heme (protoheme IX) is oxidized by heme oxygenase at the α-position to eliminate the α-carbon bridge, resulting in cleavage of the porphyrin ring and formation of an open chain tetrapyrrolic compound, biliverdin IXα.[1] The latter is subsequently reduced to bilirubin IXα by biliverdin reductase.[2] Verdohemochrome IXα was postulated as being an intermediate in the reaction process.[3,4] Biliverdins are the final products in coupled oxidation, a term used by Lemberg et al.[3b] to describe an analogous reaction of heme in a pyridine solution in the presence of ascorbic acid under air.[5] Jackson et al.[6] and Bonnett et al.[7] observed the formation of the verdohemochromes by



(1) M = Fe(II)·Py_2

(13) M = Zn



(2) M = Fe(III)·Cl

(3) M = Fe(II)·Py_2

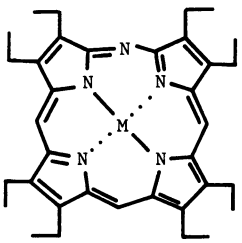
(4) M = Fe(II)·Py·CO

(5) M = Fe(II)·$(TsCH_2NC)_2$

(14) M = Zn

Py = pyridine

$TsCH_2NC$ = p-$CH_3C_6H_4SO_2CH_2NC$



(6) M = Fe(III)·Cl

(7) M = 2H

Scheme 1.

oxygenation of the corresponding oxyporphyrins; their results, thus, supported Lemberg's proposal[5a] for the formation of verdohemochromes. Lagarias[8] and Hirota and Itano[9] elucidated the structure of octaethylhemochrome, which was obtained by the coupled oxidation of octaethylheme in pyridine, as bis(pyridine)iron(II) octaethyloxaporphyrin.

We previously prepared verdohemochrome IXα by the coupled oxidation of hemoglobin and myoglobin.[4] We recently synthesized verdohemochromes by ring closure of the corresponding biliverdins.[10] Verdohemochromes are particularly susceptible to oxidative attack at the *meso*-carbons[11] at which four pyrrole rings are linked to constitute the oxaporphyrin macrocycle. Furthermore, the oxonium bridge of verdohemochromes are easily subjected to hydrolysis to open the porphyrin ring, resulting in the formation of biliverdins.[12] On successive treatment with KOH–MeOH and excess mineral acid, verdohemochrome IXα undergoes hydrolysis and demetallation to give biliverdin IXα. This reaction is accompanied by autoxidation of the chromophore to give a small amount of pink compounds. It was thought that verdohemochrome IXα gave many degradation products because of its asymmetric structure. We previously reported the oxidative degradation of octaethylverdohemochrome, a symmetric molecule, as a model compound to obtain various tripyrrins.[13]

In this paper, the detailed spectral studies of 5-azaprotohemin IX dimethyl ester (**2**) are reported. Furthermore, the isolation and structural investigations of demetallation products obtained by the reaction of **2** with concd HCl in acetic acid in the presence of iron(II) sulfate, and those of tripyrrin derivatives obtained by oxidative degradation of verdohemochrome IXα dimethyl ester (**1**) in ammonia under air are reported. The degradation of tripyrrin (**21**) with thallium(III) acetate under air is also reported.

Results and Discussion

We previously reported the formation of 5-azaprotohemin IX dimethyl ester (**2**) in good yield by treatment of verdohemochrome IXα dimethyl ester (**1**) with ammonia under argon.[10] This reaction was accompanied by the formation of a small amount of impurities that appeared as pink spots on thin layer chromotography (TLC). In an attempt to increase the yield of the pink compounds, **1** was treated with ammonia under air. The reaction mixture showed, after methylation, six pink spots (products I—VI) and a greenish brown spot (product VII) on TLC (CH_2Cl_2–MeOH, 98:2, v/v). The all products were isolated by column chromatography and preparative high performance liquid chromatography (HPLC).

Product VII was identified as authentic 5-azaprotohemin IX dimethyl ester (**2**) by mixed TLC, HPLC, and electronic absorption spectrophotometry, and by the ^{1}H NMR spectrum of iron(II) 5-azaprotoporphyrin IX dimethyl ester obtained by reduction of **2** with $Na_2S_2O_4$.

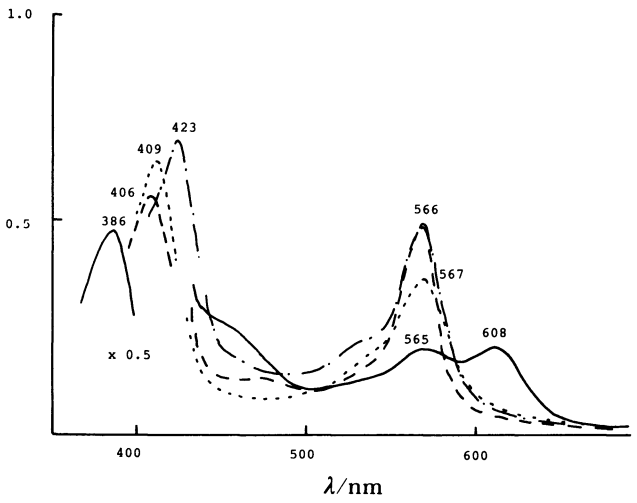


Fig. 1. Electronic absorption spectra. Spectra (1% pyridine–CH_2Cl_2, v/v) of 5-azaprotohemin IX dimethyl ester (**2**) (2.5×10^{-5} M), ——; bis(pyridine)iron(II) 5-azaprotoporphyrin IX dimethyl ester (**3**), ----; carbon monoxide co-ordination complex (**4**), ····; bis(tosylmethyl isocyanide) complex (**5**), —·—·.

The electronic absorption spectrum of compound **2** in 1% pyridine–CH_2Cl_2 showed peaks at 386, 565, and 608 nm (Fig. 1). This spectrum was not changed by the addition of excess tosylmethyl isocyanide ($TsCH_2NC$) or by the introduction of carbon monoxide. The ^{1}H NMR spectrum of **2** in $CDCl_3$ showed broadened signals (Fig. 2a). These results indicate that the oxidation state of the central metal atom of **2** is Fe(III). This was also suggested from that the addition of sodium azide resulted in the spectrum of bis(azide)-iron(III) 5-azaprotoporphyrin IX dimethyl ester with maxima at 408 and 565 nm (the data was not shown). The addition of sodium dithionite resulted in the spectrum of iron(II) 5-azaprotoporphyrin IX dimethyl ester bis(pyridine) complex **3** with maxima at 406 and 566 nm (Fig. 1). Introduction of carbon monoxide into the solution of complex **3** produced iron(II) 5-azaprotoporphyrin IX dimethyl ester carbon monoxide complex **4**. Addition of excess $TsCH_2NC$ to the solutions of **3** and **4** resulted in the electronic spectrum of iron(II) 5-azaprotoporphyrin IX dimethyl ester bis($TsCH_2NC$) complex **5**, which was identified by FAB-mass spectrum data and elemental analysis as well as the ^{1}H NMR spectral data (Fig. 2b).

Demetallation of Compound 2. Various methods for the removal of iron from hemins are known.[13] Lemberg et al.[14] and Morell et al.[15] recommended the use of the iron(II) sulfate method as the mildest and most convenient method for the preparation of

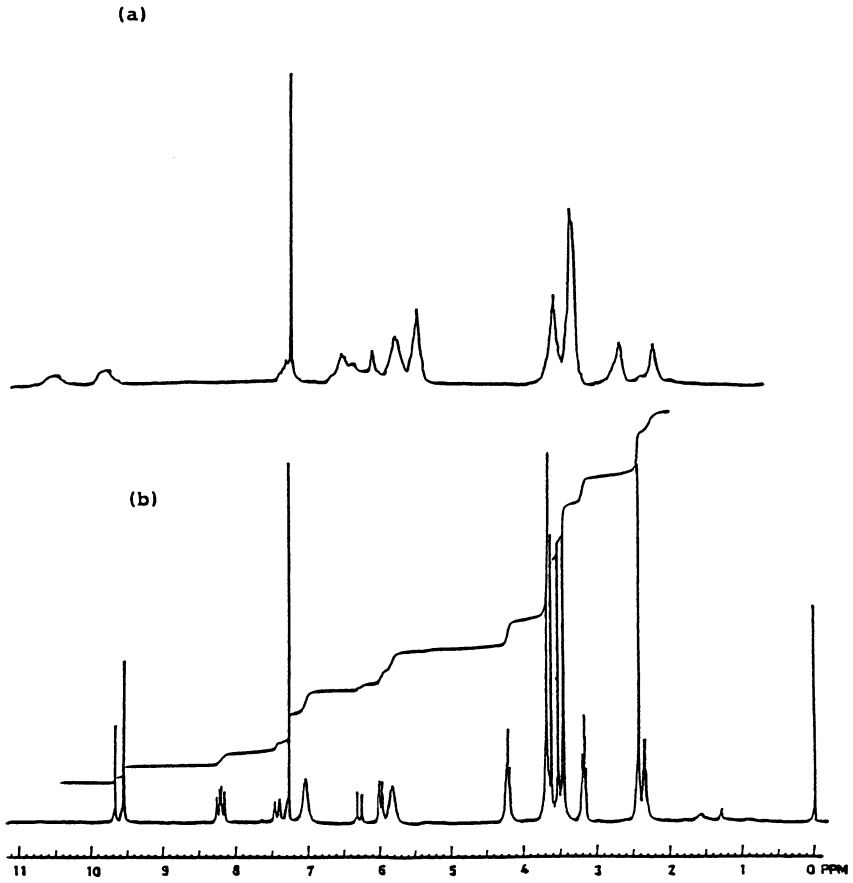


Fig. 2. 270 MHz FT ^{1}H NMR ($CDCl_3$). (a) 5-Azaprotohemin IX dimethyl ester (**2**), (b) Bis(tosylmethyl isocyanide)iron(II) 5-azaprotoporphrin IX dimethyl ester (**5**).

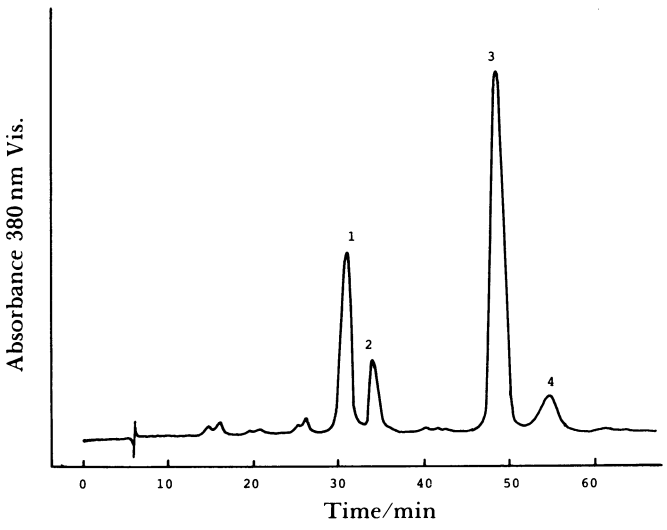


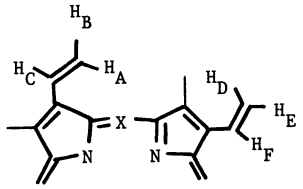
Fig. 3. High-performance liquid chromatogram of a reaction mixture obtained by the reaction of **1** with concd HCl in acetic acid in the presence of $FeSO_4$. Condition: column, SSC-ODS-H-4251, 10ϕ×250 mm; mobile phase, benzene–MeOH (1:99, v/v); visible 380 nm; flow rate, 1.0 ml min^{-1}; chart speed, 0.5 cm min^{-1}.

protoporphyrin having a labile vinyl side chain. We previously reported that the removal of iron from octaethylazahemin **6** by treatment with concd HCl in acetic acid in the presence of iron(II) sulfate gave a single product, octaethylazaprophyrin (**7**), in good yield.[10] However, compound **2** gave a mixture composed for the most part of four azaporphyrin derivatives, **8**—**11**, by the same method. Figure 3 illustrates the separation of the four products by analytical HPLC with use of isocratic elution. The products were isolated by preparative HPLC.

All products **8**—**11** showed the proton signals of three *meso*-methine groups and two imino groups in their ^{1}H NMR spectra (Table 1). In addition, the major product **8** showed the proton signals of two vinyl groups. The FAB-mass spectrum of compound **8** showed a molecular ion peak at *m/z* 591. These results suggest that compound **8** is 13,17-bis[2-(methoxycarbonyl)ethyl]-3,8-divinyl-2,7,12,18-tetramethyl-5-azaporphyrin.

Product **9** showed the proton signals of two ethyl groups as well as those of three *meso*-methine groups and two imino groups, but no vinyl protons in its ^{1}H NMR spectrum. These results indicate that

Table 1. ^{1}H NMR Data (δ in $CDCl_3$, Multiplicity and J/Hz in Parentheses)

	8	**9**	**10**	**11**
meso-H	9.57, 9.57, 9.40	10.01, 9.85, 9.84	9.96, 9.90, 9.76	10.06, 9.97, 9.86
H_A	7.45 (dd, 17.6, 2.6)	—	—	—
H_B	6.15 (dd, 11.7, 2.6)	—	—	—
H_C	8.17 (dd, 17.6, 11.7)	—	—	—
H_D	6.35 (dd, 18.0, 1.5)	—	6.40 (dd, 18.0, 1.4)	—
H_E	6.13 (dd, 11.4, 1.5)	—	6.18 (dd, 12.0, 1.4)	—
H_F	8.07 (dd, 18.0, 11.4)	—	8.15 (dd, 18.0, 12.0)	—
CH_3 on Pyrrole	3.61, 3.45, 3.38, 3.34	3.36, 3.56, 3.54, 3.52	3.61, 3.49, 3.48, 3.46	3.69, 3.57, 3.56, 3.52
>-CH_2	4.17 (4H, m)	4.31 (4H, t, 7.5)	4.27 (4H, t, 6.2)	4.35 (2H, t, 7.8), 4.30 (2H, t, 7.8)
-CH_2CO-	3.07 (4H, m)	3.22 (4H, t, 7.5)	3.18 (4H, t, 6.2)	3.23 (4H, t, 7.8)
OCH_3	3.61, 3.61	3.64, 3.64	3.63, 3.62	3.65, 3.63
NH	−3.81 (2H)	−2.73 (2H)	−3.08 (2H)	−2.73 (2H)
Others	 X=O$^+$, N, CH	3.97 (4H, q, 7.6, -$C\underline{H}_2CH_3$×2), 1.81 (6H, t, 7.6, -$CH_2C\underline{H}_3$×2)	8.34 (1H, d, 8.8, OH, exchangeable with D_2O), 6.13 (1H, m, changed to q, after addition of D_2O, -$C\underline{H}$(OH)CH_3) 2.16 (3H, d, 6.6, -CH(OH)$C\underline{H}_3$)	6.46 (1H, q, 6.6, -$C\underline{H}$(OH)CH_3) 3.39 (2H, q, 7.3, -$C\underline{H}_2CH_3$) 2.18 (3H, d, 6.6, -CH(CH)$C\underline{H}_3$) 1.83 (3H, t, 7.3, -$CH_2C\underline{H}_3$)

	5	**12**	**13**	**14**
meso-H	9.96, 9.54, 9.54	9.98, 9.98, 9.85, 9.85	9.28, 9.05, 8.97	9.04, 8.74, 8.73
H_A	7.42 (dd, 17.5, 2.6)	6.31 (dd, 18.0, 2.5)	6.69 (d, 17)	7.40 (dd, 17.8, 2.0)
H_B	5.99 (dd, 10.4, 2.6)	6.14 (dd, 11.4, 2.5)	6.00 (d, 9)	6.12 (dd, 11.9, 2.0)
H_C	8.20 (dd, 17.5, 10.4)	8.18 (dd, 18.0, 11.4)	7.35 (dd, 17, 9)	8.08 (dd, 17.8, 11.9)
H_D	6.28 (dd, 17.6, 1.5)	6.30 (dd, 17.6, 2.5)	6.17 (d, 17)	6.34 (dd, 17.8, 1.3)
H_E	6.00 (dd, 11.9, 1.5)	6.13 (dd, 11.4, 2.5)	6.10 (d, 9)	6.11 (dd, 11.5, 1.3)
H_F	8.22 (dd, 17.6, 11.9)	8.16 (dd, 17.6, 11.4)	7.58 (dd, 17, 9)	8.00 (dd, 17.8, 11.5)
CH_3 on Pyrrole	3.61, 3.52, 3.45, 3.45	3.59, 3.58, 3.53, 3.52	3.64, 3.09, 3.06, 3.03	3.53, 3.33, 3.24, 3.15
>-CH_2	4.21 (4H, t, 7.5)	4.31 (4H, t, 7.7)	3.85 (4H, t, 6.5)	3.98 (2H, t, 7.7) 3.95 (2H, t, 7.7)
-CH_2CO-	3.18 (4H, t, 7.5)	3.21 (4H, t, 7.7)	3.02 (4H, t, 6.5)	2.87 (2H, t, 7.7), 2.86 (2H, t, 7.7)
OCH_3	3.68, 3.68	3.65, 3.65	3.65, 3.65	3.57, 3.56
NH	—	−4.09 (2H)	—	—
Others	7.05 (4H, broad s, *ortho*-H on $TsCH_2NC$) 5.38 (4H, broad s, *meta*-H on $TsCH_2NC$) 2.42 (CH_3 on $TsCH_2NC$) 2.34 (CH_2 on $TsCH_2NC$)			

compound **9** is 3,8-diethyl-13,17-di[2-(methoxycarbonyl)ethyl]-2,7,12,18-tetramethyl-5-azaporphyrin. This conclusion was supported by the FAB-mass spectrum, which showed a molecular ion peak at m/z 611.

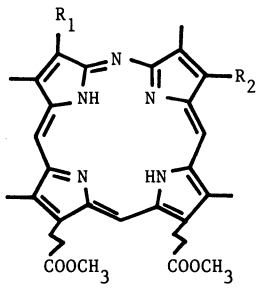
Product **10** showed the proton signals of one vinyl group and one 1-hydroxyethyl group (-CH(OH)CH_3) as well as those of three *meso*-methine groups and two imino groups. The FAB-mass spectrum of **10** showed a molecular ion peak at m/z 609. These results indicate that one of two vinyl groups of compound **8** is hydrated to obtain compound **10a** or **10b** during demetallation. For an elucidation of the structure of compound **10**, chemical shifts of vinyl protons in

compound **10** were compared with those of porphyrin derivatives **5**, **8**, **12**, and **13** in the ^{1}H NMR spectra. In this discussion, all vinyl protons on both vinyl groups at C-3 and C-8 are lettered A—F (Table 1).

The porphyrin derivatives **5**, **8**, **12**, and **13** had two vinyl groups at C-3 and C-8. In the partial structure (Table 1), when the bridge-head atom X was nitrogen (N) or oxonium (O^+), the H_A protons (δ 7.42 in **5**, 7.45 in **8**, and 6.69 in **13**) had lower chemical shifts than the other terminal vinyl protons, H_B, H_D, and H_E (δ 6.0—6.4) (Table 1). When X was the methine group (CH), as in compound **12**, all terminal vinyl protons (H_A, H_B, H_D, and H_E) in **12** appeared at δ 6.1—6.3, close to the chemical shifts of H_B, H_D, and H_E protons of compounds **5**, **8**, and **13**. These results suggest that the H_A-proton on the vinyl group at C-3 in compounds **5**, **8**, or **13** is favorably located close to the adjacent hetero atom and appears at lower field in the ^{1}H NMR spectra. Compound **12** had no bridge-head hetero atom on the porphyrin ring, therefore all terminal vinyl protons (H_A, H_B, H_D, and H_E) of compound **12** resulted in similar shifts (δ 6.1—6.3). H_D and H_E protons of the vinyl group at C-8 of compounds **5**, **8**, and **13** were located too far from the bridge-head hetero atoms and the chemical shifts of those protons (δ 6.0—6.4) were close to those to the terminal vinyl protons (H_A, H_B, H_D, and H_E) (δ 6.1—6.3) of compound **12**. In the ^{1}H NMR spectrum of compound **10** which had one vinyl group, two terminal vinyl protons appeared at δ 6.40 and 6.18, close to the chemical shifts of the terminal vinyl protons (H_D, δ 6.35 and H_E, 6.13) on the vinyl group at C-8 of compound **8**. Therefore the terminal vinyl protons appeared at δ 6.40 and 6.18 were assignable to H_D and H_E proton, respectively, on the vinyl group at C-8 of compound **10a**. These results suggest that compound **10** is 13,17-bis[2-(methoxycarbonyl)ethyl]-3-(1-hydroxyethyl)-2,7,12,18-tetramethyl-8-vinyl-5-azaporphyrin (**10a**).

Product **11** showed the proton signals of the 1-hydroxyethyl group and the ethyl group, but not those of the vinyl group. Either structure **11a** or structure **11b** was assignable to compound **11**. Though the exact structure of compound **11** was not determined, structure **11a** seems more likely because the vinyl group at C-3 in compound **8** would be more readily hydrated than that at C-8.

Electronic absorption spectra of compounds **8**—**11**



(8) $R_1 = R_2 = -CH=CH_2$

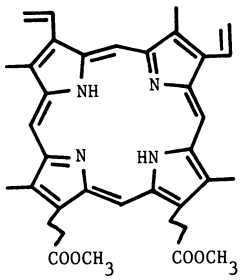
(9) $R_1 = R_2 = -CH_2CH_3$

(10a) $R_1 = -CH(OH)CH_3$, $R_2 = -CH=CH_2$

(10b) $R_1 = -CH=CH_2$, $R_2 = -CH(OH)CH_3$

(11a) $R_1 = -CH(OH)CH_3$, $R_2 = -CH_2CH_3$

(11b) $R_1 = -CH_2CH_3$, $R_2 = -CH(OH)CH_3$



(12)

Scheme 2.

Table 2. Electronic Absorption Spectra of 5-Azaporphyrins in CH_2Cl_2

Compound	λ_{max}/nm (ε_{mM})					
	Soret band	V	IV	III	II	I
8	389(138.0)	517(11.5)	548(25.7)	570(12.5)	625(26.8)	670(10.8)
9	381(151.5)	509(9.7)	540(24.4)	562(10.6)	616(25.4)	661(11.1)
10	375(145.2)	500(8.4)	534(25.7)	560(10.3)	610(25.2)	651(3.3)
11	376(142.6)	502(9.1)	535(26.4)	559(9.3)	611(25.9)	650(1.2)

in CH_2Cl_2 are listed in Table 2. All spectra showed five satellite bands, numbered I to V, as well as Soret band, respectively. The molar extinction of each band decreased with decrease in number of vinyl groups. Hypochromic shifts in the Soret bands (389→381→375 and 376 nm) corresponded to the number of vinyl groups, two in **8**, one in **10**, and none in **9** and **11**. Similar hypochromic shifts were observed in bands I—V. The extinction coefficients of band II and IV were greater than those of bands I, III, and V in all spectra. The extinction coefficients of band I of compounds **9** and **11**, which had no vinyl group, were especially small.

Preparation of Zinc Azaporphyrin (14). Iron azaprotoporphyrin complex **2** was easily obtained from the corresponding verdohemochrome **1** by treatment with ammonia. However, the reaction of zinc 5-oxaprotoporphyrin dimethyl ester **13** with ammonia, followed by methylation, gave only an open chain tetrapyrrole compounds, biliverdin IXα dimethyl ester **15**,[10,16,17] by hydrolysis but not zinc azaporphyrin complex **14**. Zinc 5-azaprotoporphyrin IX dimethyl ester **14** was prepared by the reaction of azaprotoporphyrin IX dimethyl ester **8** with $Zn(OAc)_2$ in methanol. The ^1H NMR spectra (Table 1) of the zinc complex **14** was similar to that of **8**; the H_A-proton (δ 7.40) was shifted to lower field than the other terminal vinyl protons (H_B, H_D, and H_E) (δ 6.11—6.34). The FAB-mass spectrum showed fragment ion peaks at *m/z* 653, 655, and 657, which correspond to the cations $C_{35}H_{35}N_5O_4{}^{64}Zn^+$, $C_{35}H_{35}N_5O_4{}^{66}Zn^+$, and $C_{35}H_{35}N_5O_4{}^{68}Zn^+$, respectively.

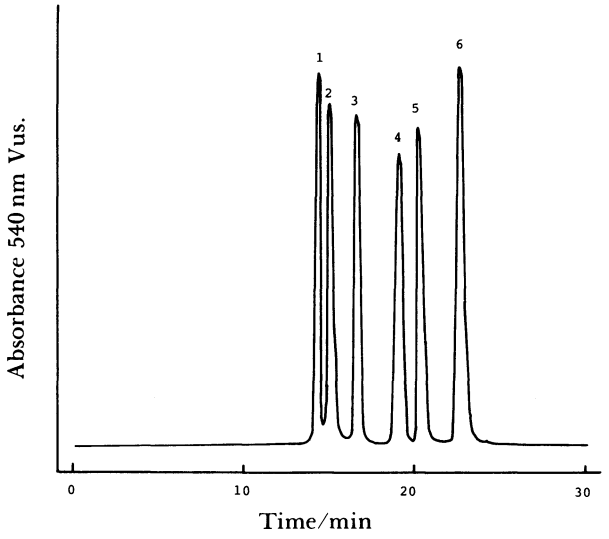


Fig. 4. High-performance liquid chromatogram of the degradation products. Condition: column, LiChrosorb SI-60, 4.6ϕ×250 nm; mobile phase, CH_2Cl_2-MeOH, 97.5:2.5 (v/v); visible, 540 m,; flow rate, 1.0 ml min^{-1}; chart speed, 0.5 cm min^{-1}. Injection was at time zero. Peak 1, **16**; 2, **17**; 3, **18**; 4, **20**; 5, **19**; 6, **21**.

Structures of Tripyrrins 16—21. Products (I—VI) were isolated by column chromatography and preparative HPLC. Figure 4 shows HPLC chromatogram for the isolated products (I—VI). The products (I—VI) showed the proton signals of two methine groups, three methyl groups, two 2-(methoxycarbonyl)ethyl groups, and one vinyl group in their ^1H NMR spectra (Table 3). These results indicate that the products (I—VI) are tripyrrin derivatives which are derived by the loss of one of the terminal pyrrole rings from the tetrapyrrole derivatives, verdohemochromes and biliverdins by oxidative degradation.[18,19] In addition to these signals, products I and II showed the singlet of the methoxycarbonyl group (δ 3.98 in I and 3.98 in II, respectively). The EI mass spectra of both I and II showed molecular ion peaks at *m/z* 535 (relative intensity: 38.4% in I and 92.7% in II, respectively). These results suggest that products I or II is either of the two tripyrrins (**16** or **17**).

Products III and V showed the proton signals of the dimethyl acetal group [δ 5.61 ($-C\underline{H}(OCH_3)_2$) and 3.38 ($-CH(OC\underline{H}_3)_2$) in III, and 5.61 ($-C\underline{H}(OCH_3)_2$ and 3.38 ($-CH(OC\underline{H}_3)_2$) in V, respectively] as well as those of

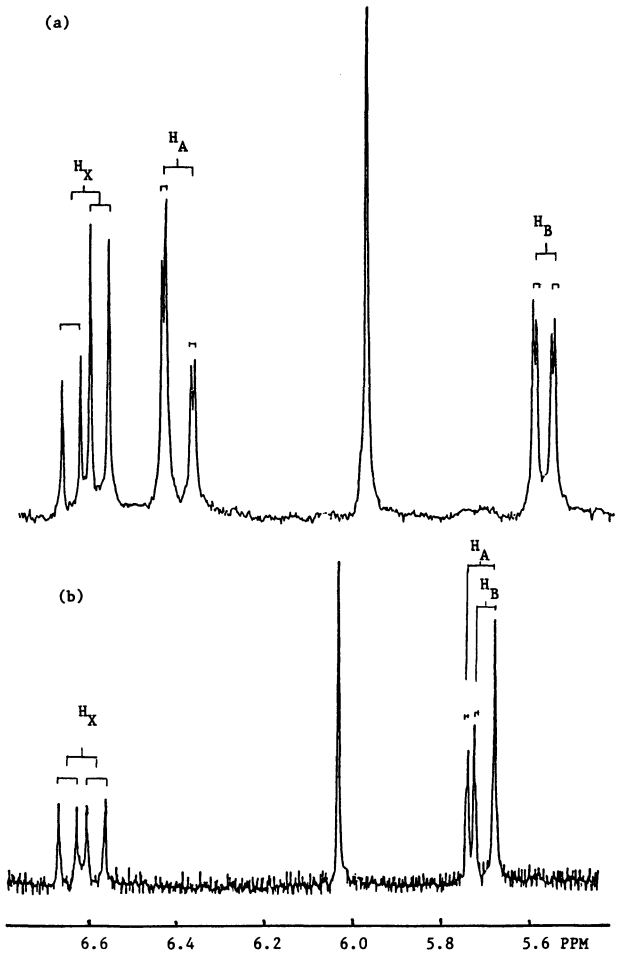


Fig. 5. Partial ^1H NMR spectra (downfield) of compounds **16** and **17**. (a), **16**; (b), **17**.

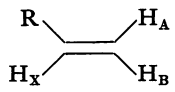
two *meso*-methine groups, two 2-(methoxycarbonyl)-ethyl groups, and one vinyl group in their ^{1}H NMR spectra. These results suggest that product III or V is either of the two tripyrrins **18** or **19**. This conclusion was also suggested by the EI mass spectra of III and V, which showed molecular ion peak at *m/z* 551.

The ^{1}H NMR spectra of products IV and VI showed the proton signals of the aldehyde group (δ 9.83 in IV and 9.82 in VI, respectively) as well as those of two *meso*-methine groups, two 2-(methoxycarbonyl)ethyl groups, and one vinyl group. The EI mass spectra of IV and VI showed molecular ion peak at *m/z* 505. These results suggest that product IV or VI is either of two tripyrrins **20** or **21**.

The exact structures of these products **16—21** were elucidated by comparing the chemical shifts of the vinyl protons of the products **16—21** with those of porphyrin derivatives **5**, **8**, and **13**, and those of biliverdin derivatives **15**, and **22—24** in the ^{1}H NMR spectra (Tables 3 and 4, and Fig. 5). As mentioned

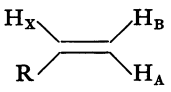
Table 3. ^{1}H NMR Data for Tripyrrins (δ in $CDCl_3$, Multiplicity and *J*/Hz in Parentheses)

	16	**17**	**18**	**19**
meso-H	6.85, 5.96	6.85, 6.05	6.87, 5.99	6.84, 6.06
H_A	6.39 (dd, 17.7, 2.4)	5.71 (dd, 16.9, 1.1)	6.35 (dd, 17.6, 2.2)	5.69 (dd, 17.6, 0.5)
H_B	5.56 (dd, 11.6, 2.4)	5.70 (dd, 11.7, 1.1)	5.52 (dd, 11.0, 2.2)	5.68 (dd, 11.5, 0.5)
H_X	6.60 (dd, 17.7, 11.6)	6.66 (dd, 16.9, 11.7)	6.60 (dd, 17.6, 11.0)	6.65 (dd, 17.6, 11.5)
CH_3 on pyrrole	2.34, 2.21, 2.09	2.34, 2.10, 2.08	2.20, 2.09, 2.04	2.08, 2.07, 2.04
>-CH_2-	2.94 (4H, m)	2.94 (4H, m)	2.96 (2H, t, 7.1) 2.92 (2H, t, 7.1)	2.96 (2H, t, 7.1) 2.92 (2H, t, 7.1)
-CH_2CO-	2.55 (4H, m)	2.55 (4H, m)	2.54 (4H, t, 7.1)	2.55 (4H, t, 7.1)
/\\/$COOCH_3$	3.69, 3.65	3.67, 3.65	3.67, 3.65	3.67, 3.65
NH	11.20, 9.36	11.25, 9.56	10.90, 9.33	11.20, 9.45
Others	3.98 (3H, s, >-$COOCH_3$)	3.98 (3H, s, >-$COOCH_3$)	5.61 (1H, s, -C<u>H</u>$(OCH_3)_2$) 3.38 (6H, s, -CH$(OCH_3)_2$)	5.61 (1H, s, -C<u>H</u>$(OCH_3)_2$) 3.38 (6H, s, -CH$(OCH_3)_2$)



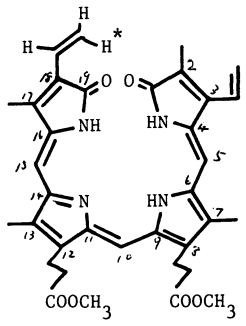
	20	**21**
meso-H	6.83, 5.95	6.84, 6.01
H_A	6.54 (dd, 17.7, 3.1)	5.70 (dd, 18.3, 0.5)
H_B	5.49 (dd, 10.4, 3.1)	5.69 (dd, 12.2, 0.5)
H_X	6.60 (dd, 17.7, 10.4)	6.63 (dd, 18.3, 12.2)
CH_3 on pyrrole	2.34, 2.19, 2.09	2.33, 2.09, 2.07
>-CH_2-	2.96 (2H, t, 7.4) 2.91 (2H, t, 7.4)	2.96 (2H, t, 7.3) 2.93 (2H, t, 7.3)
-CH_2CO-	2.57 (4H, t, 7.4)	2.57 (4H, t, 7.3)
/\\/$COOCH_3$	3.67, 3.65	3.67, 3.59
NH	11.25, 9.39	11.23, 9.59
Others	9.83 (1H, s, -CHO)	9.82 (1H, s, -CHO)

Table 4. Chemical Shifts (δ in $CDCl_3$) for Vinyl Protons of Compounds **15** and **22—24**[a)]

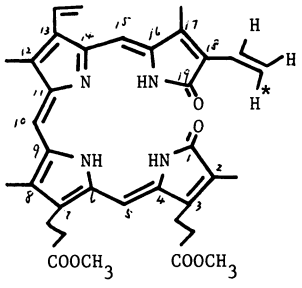


	15	**22**	**23**	**24**
Vinyl				
δ_A	6.12, 5.66	5.45, 6.12	5.49, 5.40	5.66, 5.50
δ_B	5.43, 5.64	5.44, 5.31	5.44, 5.38	5.64, 5.44
δ_X	6.50, 6.62	6.68, 6.49	6.73, 6.60	6.61, 6.76

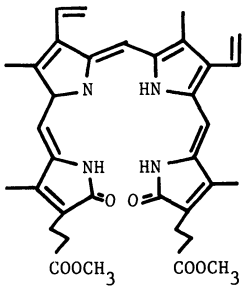
a) These data were obtained by Rasmussen et al.[20)]



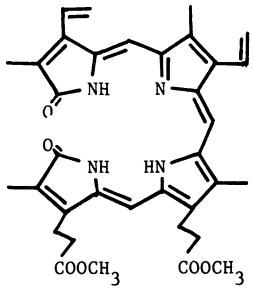
(15)



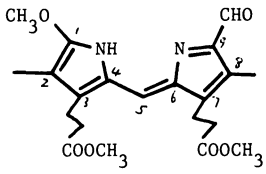
(22)



(23)



(24)



(27)

Scheme 3.

above, porphyrins **5**, **8**, and **13** have two vinyl groups at C-3 and C-8. The H_A-protons on the vinyl groups at C-3 in compounds **5**, **8**, and **13** appeared at lower field than the other terminal vinyl protons (H_B on vinyl group at C-3, and H_D and H_E on vinyl group at C-8). This conclusion will be applied to the assignment of the vinyl groups of biliverdins **15** and **22**—**24**.

Rasmussen et al.[17] reported the ^1H NMR spectra of four biliverdin dimethyl ester isomers **15** and **22**—**24**, of the IX series, but did not report detailed assignment of the vinyl protons. All vinyl groups of compounds **23** and **24**, and the vinyl groups at C-3 of **15** and at C-13 of **22** were located apart from the carbonyl groups of the terminally located pyrrole rings; all terminal vinyl protons (H_A and H_B in Table 4) of these vinyl groups appeared at δ 5.38—5.66. On the other hand, the protons marked with an asterisk (*) on the vinyl group at C-18 of **15** and that at C-18 of **22** were located close to the carbonyl groups (at C-19). These protons (H*) of **15** and **22**, therefore, appeared at lower fields (δ 6.12) in the ^1H NMR spectra of these compounds (Table 4).

Six tripyrrin derivatives **16**—**21** were divided into two groups (A and B) (Table 5) by comparison of the chemical shifts of the terminal vinyl protons (H_A and H_B) in their ^1H NMR spectra (Table 3). Figure 6 showed the partial ^1H NMR spectra (downfield) of compounds **16** and **17**. A comparison of the spectra shows the H_A-proton at a lower field (δ 6.39) than the H_B-proton (δ 5.56) in compound **16**. On the other hand, both the H_A and H_B protons of **17** showed nearly the same chemical shift at δ 5.71 and 5.70, respectively. The results indicate that the vinyl group of compound **16** is located close to the carbonyl group at C-1, and that the vinyl group of compound **17** is located at C-3 at a greater distance from the carbonyl group. Therefore, products I and II are identified as 14-methoxycarbonyl-8,12-bis[2-(methoxycarbonyl)-ethyl]-3,7,13-trimethyl-2-vinyltripyrrin-1-one (**16**) and 14-methoxycarbonyl-8,12-bis[2-(methoxycarbonyl)ethyl]-2,7,13-trimethyl-3-vinyltripyrrin-1-one (**17**), respec-

tively.

The same comparisons were done between products III and V, and also between IV and VI. The H_A-protons of products III and V appeared at lower fields (δ 6.35 in III and 6.54 in V, respectively). H_A-protons of V and VI were observed at δ 5.69 in IV and 5.70 in VI, respectively; these chemical shift values were close to those of the H_B-protons (δ 5.68 in IV and 5.69 in VI). Thus, products III—VI were identified as 8,12-bis[2-(methoxycarbonyl)ethyl]-3,7,13-trimethyl-1-oxo-2-vinyltripyrrin-14-carbaldehyde dimethyl acetal (**18**), 8,12-bis[2-(methoxycarbonyl)ethyl]-3,7,13-trimethyl-1-oxo-2-vinyltripyrrin-14-carbaldehyde (**20**), 8,12-bis[2-(methoxycarbonyl)ethyl]-2,7,13-trimethyl-1-oxo-3-vinyltripyrrin-14-carbaldehyde dimethyl acetal (**19**), and 8,12-bis[2-(methoxycarbonyl)-ethyl]-2,7,13-trimethyl-1-oxo-3-vinyltripyrrin-14-carbaldehyde (**21**), respectively. Compounds **16**, **18**, and **20** in group A (Table 5) accordingly were products of the loss of pyrrole ring B from compound **1** by oxidation degradation, and compound **17**, **19**, and **21** were products of the loss of ring A from **1**.

The electronic absorption spectra of compounds **16—21** are listed in Table 6. In comparisons of the spectra of compounds in group A with those of compounds in group B (Tables 5 and 6), individual bands of compound **16** had higher wavelength by 5—6 nm than the corresponding bands of compound **17**, 543, 509, 475sh, and 327 nm in **16** and 537, 504, 470sh, and 322 nm in **17**. The same results were obtained

Table 5. Structures of Tripyrrins

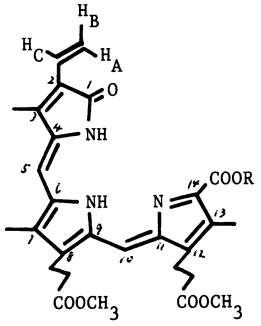
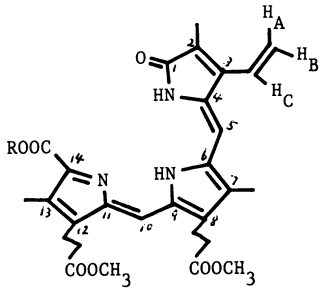
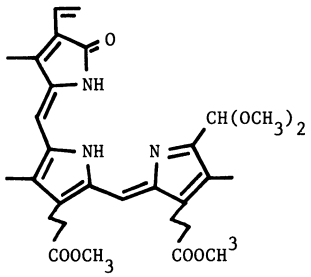
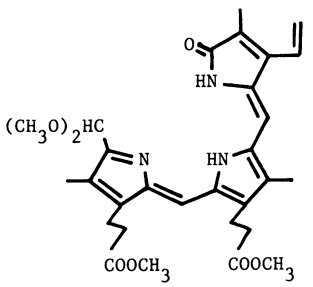
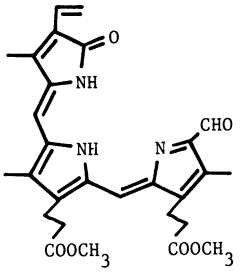
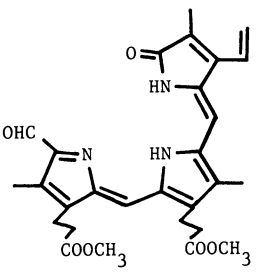
Group A	Group B
 (16) R = CH_3 (25) R = H	 (17) R = CH_3 (26) R = H
 (18)	 (19)
 (20)	 (21)

Table 6. Electronic Absorption Spectra of Degradation Products in CH_2Cl_2

	Group A							
	Free base λ_{max}/nm (ε_{mM})				Zinc complex[a] λ_{max}/nm (ε_{mM})			
16	543 (16.2)	509 (14.8)	475 sh[b] (7.8)	327 (25.9)	629 (22.2)	583 (12.0)	545 sh (5.6)	351 (28.7)
18	549 (14.4)	515 (14.5)	480 sh (8.4)	330 (32.2)	634 (13.4)	588 (10.0)	550 sh (5.2)	352 (31.7)
20	548 (15.6)	515 (15.7)	480 sh (9.2)	330 (37.0)	635 (14.9)	558 (10.6)	550 sh (5.3)	351 (35.5)

	Group B							
	Free base λ_{max}/nm (ε_{mM})				Zinc complex λ_{max}/nm (ε_{mM})			
17	537 (15.9)	504 (15.1)	470 sh (8.4)	322 (21.4)	624 (22.7)	578 (13.4)	540 sh (5.8)	345 (20.6)
19	543 (16.4)	510 (17.2)	475 sh (10.2)	325 (32.4)	630 (15.9)	585 (12.3)	547 sh (6.1)	345 (32.8)
21	543 (14.7)	510 (15.1)	475 sh (10.1)	325 (34.5)	630 (16.5)	585 (14.0)	547 sh (7.1)	341 (38.8)

a) 50 μl of zinc acetate (0.85 M) in MeOH was added to each sample cuvette. b) sh=shoulder.

Table 7. ^{13}C Chemical Shifts ($CDCl_3$) for Compounds **20** and **21** at 67.8 Mz (Downfield from Me_4Si)

	meso-C (C-5, 10)	$CH_2=\underline{C}H-$	$\underline{C}H_2=CH-$	$\underline{C}H_2CH_2CO$	$CH_2\underline{C}H_2CO$	$COO\underline{C}H_3$	$\underline{C}OOCH_3$	CH_3	CHO	C-1	Ring carbon atoms except C-1
20	96.1	122.5	125.4	19.7	34.9	51.6	172.8	9.8	177.7	169.8	145.6, 143.4, 139.7, 134.9,
	114.3			19.2			172.7	9.4			133.3, 133.2, 130.2, 128.7,
								8.5			128.3
21	96.8	123.0	126.1	19.8	35.0	51.7	173.0	9.8	178.0	169.1	153.3, 144.6, 143.5, 139.9,
	114.3			19.2			171.1	8.6			135.1, 133.3, 133.2, 130.9, 130.3, 128.7

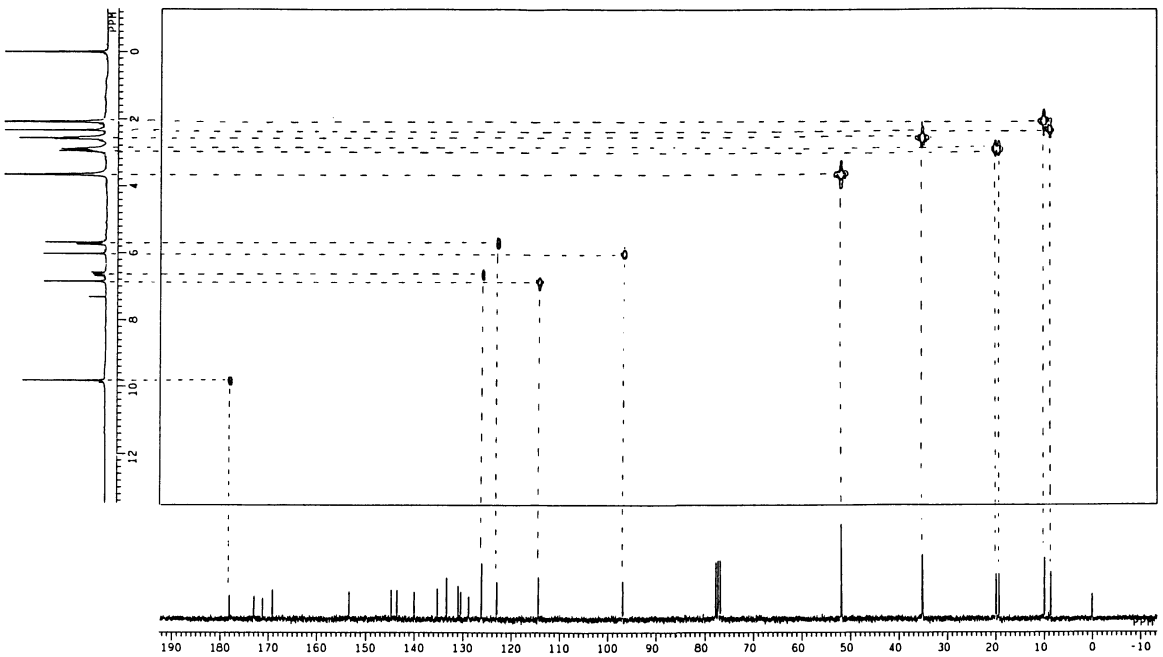


Fig. 6. Two dimensional (^1H–^{13}C) NMR spectrum of **21**.

between **18** and **19**, and between **20** and **21**.

The ^{13}C NMR spectra of compounds **20** and **21**, which were major products by oxidative degradation of **1**, are given in Table 7. The carbon signals were assigned by the single pulse hetero decoupling method (with NOE) and by the two dimentional (^1H–^{13}C) NMR spectra (the 2D NMR spectrum of **21** is shown in Fig. 6).

Degradation of Compound 1 on TLC under Air. Verdohemochrome IXα dimethyl ester **1** showed a single band at R_f 0.53 on preparative TLC (CH_2Cl_2–MeOH, 9:1, v/v). When the TLC plate was allowed to stand for a week under air, the color of the green band changed to pink. The band was scraped off and extracted. The extracts showed two major spots (R_f 0.36 and 0.32) and two minor spots (R_f 0.06 and 0.04) on TLC (CH_2Cl_2–MeOH, 98:2, v/v). The major products with R_f 0.36 and 0.32 were identified as compounds **20** and **21**, respectively, by mixed TLC, HPLC, and ^1H NMR spectra. The minor products with R_f 0.06 and 0.04 were unstable. The products showed the ^1H NMR spectra similar to those of **20** and **21**, respectively, except for absence of the signal of the methoxyl methyl proton. After methylation, the product with R_f 0.06 gave compound **20**, and that with R_f 0.04 gave compound **21**. These results suggest that the minor products with R_f 0.06 and 0.04 on TLC are compounds **25** and **26**, respectively.

Oxidative Degradation of 8 with Tl(OAc)$_3$. We previously reported the oxidative degradation of octaethylbiliverdin with thallium(III) acetate under air.[18] Further oxidation of the major tripyrrin derivative **21** was studied. The mixture obtained by the reaction of **21** with Tl(OAc)$_3$ in CH_2Cl_2–MeOH (9:1) under air showed two yellow spots (R_f 0.42 and 0.31) on TLC (benzene–acetone, 9:1, v/v). The compound with R_f 0.42 was isolated by column chromatography, but the compound with R_f 0.31 was so unstable that its isolation was unsuccessful. The ^1H NMR spectrum of the compound with R_f 0.42 on TLC showed the proton signals of an aldehydo group (δ 9.68) and a metoxyl group (δ 4.13) as well as those of two 2-(methoxycarbonyl)ethyl groups and two methyl groups but no vinyl group. The EI mass spectrum showed a molecular ion peak at m/z 402 and fragment ion peaks at m/z 387 (M^+-CH_3), 371 (M^+-OCH_3), and 343 ($M^+-COOCH_3$). These results suggest that the product is 3,7-bis[2-(methoxycarbonyl)ethyl]-2,8-dimethyl-1-methoxy-5-pyrromethene-9-carbaldehyde (**27**).

Thus, tripyrrin derivatives **16—21** were easily obtained from verdohemochrome IXα dimethyl ester **1** by oxidative degradation. Tripyrrin **21** was also oxidatively degraded to produce pyrromethene derivative **27**. Schaefer et al.[20] reported that cytochrome P-450 and free ferriprotoporphyrin IX were destroyed to obtain propentdyopents (dipyrrole derivatives) by NADPH-P-450 reductase in the presence of NADPH and O_2. In the present study, though the product **27** obtained by the reaction of tripyrrin **21** with Tl(OAc)$_3$ under air is different from those obtained by Shaefer et al. because of the difference of the reaction methods, the possibility of the presence of new metabolic path way for heme, heme→verdohemochrome→tripyrrins→dipyrrole derivatives, is offered.

Experimental

Materials. Verdohemochrome IXα dimethyl ester (**1**) was obtained by the method of Saito and Itano.[4] All chemicals and solvents were of reagent grade and were obtained from commercial sources.

Measurements. Electronic absorption spectra were recorded with a Hitachi Model 100-50 spectrophotometer. EI and FAB mass spectra were obtained with JEOL JMS-DX 300 mass spectrometer. High resolution mass spectra were obtained with JEOL OISG-2 mass spectrometer. ^1H NMR spectra at 270 MHz and ^{13}C NMR spectra at 67.8 MHz of samples in $CDCl_3$ solution containing Me_4Si were recorded with a JEOL JNM-GX 270 FT NMR spectrometer. The Shimadzu LC-6A liquid chromatograph, equipped with a Shimadzu system controller SCL-6A, a Shimadzu SPD-6AV UV-VIS spectrophotometric detector, and a Shimadzu C-R3A Chromatopac, were used for analytical high-performance liquid chromatography (HPLC), and were further equipped with an SSC autoinjector 6310 and an SSC fraction collector 6320 (Senshu Scientific Co. LTD.) for preparative HPLC. Analytical thin layer chromatography (TLC) and preparative TLC were performed on DC-Alufolien Kieselgel 60 HF_{254} (Merck) and Kieselgel 60 HF_{254} (Merck), respectively. Wacogel C-200 was used for column chromatography. Melting points were determined with a Yanako micro melting apparatus.

Reaction of 1 with Ammonia. Verdohemochrome IXα dimethyl ester (**1**) (460 mg, 502 μmol) was dissolved in the solution of pyridine (5 ml) and concd ammonia (50 ml) and allowed to stand under air overnight. The reaction mixture was poured into ice water (100 ml) and extracted with CH_2Cl_2 (150 ml×3). The combined organic extracts were washed with H_2O, dried over Na_2SO_4, and filtered. The filtrate was dissolved in 5% H_2SO_4–MeOH (150 ml) and allowed to stand overnight. The reaction mixture was poured into ice water (200 ml) and extracted with CH_2Cl_2 (200 ml×3). The combined organic extracts were washed with H_2O, dried over Na_2SO_4, and filtered. The filtrate was evaporated to give a residue which showed six pink spots (I, R_f 0.49; II, 0.44; III, 0.41; IV, 0.38; V, 0.34; and VI, 0.30) and a greenish brown spot (VII, 0.07) on TLC (CH_2Cl_2–MeOH, 98:2, v/v). The residue was dissolved in CH_2Cl_2 (5 ml) and subjected to column chromatography (CH_2Cl_2–MeOH, gradient up to 10%, v/v). Product VII was isolated, and products I—VI were partially purified. The partially purified products were subjected to preparative HPLC (LiChrosorb SI-60, 10φ, 250 mm, solvent system: CH_2Cl_2–MeOH, 97.5:2.5, v/v).

Product VII, yield 93.3 mg, 27.3%, was identified as 5-azaprotohemin IX dimethyl ester (**2**) by mixed TLC and electronic absorption spectrum, and the ^1H NMR spectrum of iron(II) bis(tosylmethyl isocyanide)-5-azaprotoheme IX dimethyl ester derived from **2** by the method of Saito and Itano.[10]

Product I: Yield 8 mg (3.0%), EI MS (70 eV) *m/z* (rel intensity) 535 (M^+, 38.4), 504 (M^+-OCH_3, 9.5), 503 (M^+-HOCH_3, 11.3), 462 (12.0), 448 (5.4), 444 (11.3), 430 (15.5), 416 (10.8), 388 (6.0), 239 (13.3), 237 (9.5), 234 (8.4), 235 (7.1), 183 (10.0), 178 (19.6), 85 (13.1), 83 (11.1), 71 (16.2), 69 (13.4), 57 (32.5), 44 (41.1), 43 (26.9), 32 (24.2), 28 (100). Found: *m/z* 535.2326. Calcd for $C_{29}H_{33}N_3O_7$: M, 535.2316.

Product II: Yield 15 mg (5.6%), EI MS (70 eV) *m/z* (rel intensity) 535 (M^+, 92.7), 504 (M^+-OCH_3, 22.5), 503 (M^+-HOCH_3, 9.5), 476 ($M^+-COOCH_3$, 6.6), 462 (13.9), 449 (10.0), 448 (33.8), 444 (20.0), 430 (19.9), 416 (28.0), 388 (14.8), 375 (11.0), 165 (8.9), 57 (11.4), 43 (9.6), 32 (18.5), 28 (100). Found: *m/z* 535.2303. Calcd for $C_{29}H_{33}N_3O_7$: M, 535.2316.

Product III: Yield 16 mg (5.8%), EI MS spectrum *m/z* (rel intensity) 551 (M^+, trace), 368 (5.1), 279 (5.6), 149 (22.8), 127 (6.4), 125 (7.2), 114 (6.7), 111 (12.9), 99 (11.9), 97 (22.5), 86 (62.0), 84 (79.9), 44 (100). Found: *m/z* 551.2631. Calcd for $C_{30}H_{37}N_3O_7$: M, 551.2629.

Product V: Yield 26 mg (9,4%), EI MS (70 eV) *m/z* (rel intensity) 551 (M^+, 5.5), 491 (5.0), 368 (6.0), 300 (9.0), 124 (5.1), 115 (12.3), 124 (17.4), 100 (41.9), 96 (35.9), 86 (17.3), 84 (22.3), 72 (96.4), 44 (48.1), 43 (100). Found: *m/z* 551.2625. Calcd for $C_{30}H_{37}N_3O_7$: M, 551.2629.

Product IV: Yield 32 mg (12.6%), EI MS (70 eV) *m/z* (rel intensity) 505 (M^+, 5.5), 418 (10.5), 358 (6.3), 181 (7.2), 148 (11.0), 136 (5.2), 122 (6.8), 108 (11.7), 94 (9.7), 57 (5.0), 44 (22.9), 43 (5.6), 32 (23.0), 28 (100). Found: *m/z* 505.2209. Calcd for $C_{28}H_{31}N_3O_6$: M, 505.2211.

Product VI: Yield 75 mg (29.6%), EI MS (70 eV) *m/z* (rel intensity) 505 (M^+, 60.4), 474 (10.0), 446 (5.3), 432 (22.5), 418 (26.1), 358 (10.8), 345 (6.6), 330 (8.9), 313 (5.5), 300 (5.0), 225 (6.1), 57 (13.6), 44 (14.0), 43 (8.9), 32 (20.6), 28 (100). Found: *m/z* 505.2215. Calcd for $C_{28}H_{31}N_3O_6$: M, 505.2211.

Demetallation of 2. To a solution of compound **2** (85 mg) in pyridine (1 ml) and acetic acid (30 ml), a solution of $FeSO_4$ (80 mg) in concd HCl (1 ml) was added under argon, and the reaction mixture was allowed to stand for 30 min. The reaction mixture was poured into ice water (50 ml) and extracted with CH_2Cl_2 (50 ml×3). The combined organic extracts were washed with water, dried over Na_2SO_4, and filtered. The filtrate was evaporated to give a residue which was dissolved in 5% H_2SO_4–MeOH (200 ml) and allowed to stand overnight. The mixture was poured into ice water (300 ml) and extracted with CH_2Cl_2 (150 ml×3). The combined organic extracts were washed with water, dried over Na_2SO_4, and filtered. The filtrate was evaporated to give a residue which was subjected to preparative HPLC (column: SSC-ODS-H-4251, 10ϕ, 250 mm; solvent system: 1% benzene–MeOH) to isolate compounds **8**—**11**. All products **8**—**11** had melting points over 300 °C.

Compound **8**: yield 33.5 mg (47.3%), FAB MS *m/z* 591 (100%). Found: C, 70.93; H, 6.31; N, 11.59%. Calcd for $C_{35}H_{37}N_5O_4$: C, 71.05; H, 6.30; N, 11.84%.

Compound **9**: yield 6.1 mg (8.6%), FAB MS *m/z* 595 (100%). Found: C, 70.47; H, 6.96; N, 11.69%. Calcd for $C_{35}H_{41}N_5O_4$: C, 70.56; H, 6.94; N, 11.76%.

Compound **10**: yield 3.4 mg (4.8%), FAB MS *m/z* 609 (100%). Found: C, 69.02; H, 6.41; N, 11.38%. Calcd for $C_{35}H_{39}N_5O_5$: C, 68.95; H, 6.45; N, 11.49%.

Compound **11**: yield 7.9 mg (10.7%), FAB MS *m/z* 611 (100%). Found: C, 68.85; H, 6.75; N, 11.22%. Calcd for $C_{35}H_{41}N_5O_5$: C, 68.72; H, 6.76; N, 11.45%.

Reaction of 13 with Ammonia. Compound **13** (20 mg) was dissolved in pyridine (1 ml) and concd aqueous ammonia (30 ml) under argon. The mixture was allowed to stand overnight, poured into ice water (150 ml), and extracted with CH_2Cl_2 (150 ml×3). The combined organic extracts were washed with water, dried over Na_2SO_4, and filtered. The filtrate was evaporated to give a residue which was dissolved in 5% H_2SO_4–MeOH (100 ml) and allowed to stand overnight. The reaction mixture was poured into ice water (200 ml) and extracted with water, dried over Na_2SO_4, and filtered. The filtrate was evaporated to give a residue which was subjected to column chromatography (benzene–acetone, gradient up to 5%) to obtain biliverdin IXα dimethyl ester (**15**), which was identified by TLC, mass spectrum, and ^1H NMR spectrum. No azaporphrin derivative was obtained by this reaction.

Preparation of 14. To a solution of compound **8** (12 mg) in CH_2Cl_2 (5 ml), a solution of zinc acetate (20 mg) in MeOH (10 ml) was added and warmed at 60 °C for 30 min. The solvent was evaporated, and the resulting residue was dissolved in CH_2Cl_2 (10 ml) and filtered and washed with CH_2Cl_2 (10 ml×3). The combined filtrates were evaporated to obtain a residue which was subjected to column chromatography (CH_2Cl_2–MeOH, gradient up to 5%) to isolate compound **14**. Found: C, 63.99; H, 5.41; N, 10.43%. Calcd for $C_{35}H_{35}N_5O_4Zn$: C, 64.13; H, 5.38; N, 10.68%. λ_{max} (CH_2Cl_2) nm (ε_{mM}) 588 (31.7), 547 (8.4), and 406 (87.1).

Iron(II) Bis(tosylmethyl isocyanide)-5-azaprotoporphyrin IX Dimethyl Ester (5). To a solution of **2** (17 mg) in pyridine (5 ml) and CH_2Cl_2 (20 ml), a solution of sodium dithionite (30 mg) in H_2O (1 ml) was added. The solution was vigorously shaken, and the mixture was washed with H_2O (20 ml×2), dried over Na_2SO_4, and filtered. The filtrate was added with pure $TsCH_2NC$ (25 mg) and subjected to column chromatography (CH_2Cl_2) to obtain compound **5**. Yield 17.5 mg (60.6%). Found: C, 61.05; H, 5.27; N, 9.14%. Calcd for $C_{53}H_{53}N_7O_8S_2Fe$: C, 61.44; H, 5.16; N, 9.46%.

Autoxidation of 1 on TLC. Compound **1** (150 mg) was dissolved in CH_2Cl_2 (1 ml) and chromatographed by prep. TLC (CH_2Cl_2–MeOH, 9:1, v/v). After development, the TLC plate was allowed to stand for a week under air at room temperature. The colored band was scraped off and extracted with CH_2Cl_2–MeOH (9:1, v/v). The extract was evaporated to give a residue, which was subjected to column chromatography (CH_2Cl_2–MeOH, gradient up to 10%) to obtain major product **20** (15.2 mg, 18.4%) and **21** (23.1 mg, 27.9%), and two minor products **25** (4.0 mg, 5.0%) and **26** (7.3 mg, 9.1%). The major porducts were identified with compounds **20** and **21**, respectively, by mixed TLC, HPLC, and ^1H NMR spectra. Though the minor products **25** and **26** were unstable, their ^1H NMR spectra were obtained. ^1H NMR ($CDCl_3$) of **25** δ=11.23—10.50 (2H, exchangeable with D_2O), 9.40 (1H, NH, exchangeable with D_2O), 6.84 (1H, s, *meso*-H), 6.59 (1H, dd, J=17.8 and 11.1 Hz, H_X), 6.38 (1H, dd, J=17.8 and 2.1 Hz, H_A), 5.96 (1H, s, *meso*-H), 5.55 (1H, dd, J=11.6 and 2.1 Hz, H_B), 3.67 and 3.65 (each 3H, s, $COOCH_3$), 2.96 (4H, $\gg$-CH_2-X2), 2.55 (4H, -CH_2CO-X2), 2.32, 2.18, and 2.07 (each 3H, s, CH_3). ^1H NMR ($CDCl_3$) of **26** δ=11.20—10.00 (2H, exchangeable with D_2O), 9.45 (1H, exchangeable with D_2O), 6.84 (1H, s, *meso*-H), 6.65 (1H, dd, J=17.8 and 11.0 Hz, H_X), 6.05 (1H, s, *meso*-H), 5.66 (1H, dd, J=17.8 and 1.4 Hz, H_A), 5.67 (1H, dd, J=11.0 and 1.4 Hz, H_B), 3.67 and 3.65 (each 3H, s, $COOCH_3$), 2.95 (4H,

>-CH_2-X2), 2.56 (4H, -CH_2CO-X2), 2.28, 2.07, and 2.04 (each 3H, s, CH_3).

Reaction 21 with Tl(OAc)$_3$. To a solution of **21** (50 mg) in CH_2Cl_2 (45 ml), a solution of thallium(III) acetate sesquihydrate (41 mg) in MeOH (5 ml) was added under air. The reaction mixture was poured into ice water (100 ml) and extracted with CH_2Cl_2 (100 ml×3). The combined organic extracts were washed with H_2O, dried over Na_2SO_4, and filtered. The filtrate was evaporated to obtain a residue which showed two yellow spots (R_f 0.42 and 0.31, benzene–acetone, 9:1, v/v). The residue was subjected to column chromatography to isolate the product **27** (22.7 mg, 57.0%) with R_f 0.42. The product with R_f 0.31 was so unstable that it could not be isolated. EI MS (70 eV) *m/z* (rel intensity) 402 (M^+, 100), 387 (14.7), 371 (17.3), 343 (16.5), 330 (8.6), 329 (38.8), 316 (10.1), 315 (47.4), 314 (25.7), 313 (61.8), 287 (8.0), 285 (8.8), 281 (7.3), 225 (11.9). λ_{max} (CH_2Cl_2) (ε_{mM}) 421 (30.1), 401 (27.8), and 380sh (16.0). ^{1}H NMR ($CDCl_3$) δ=11.75 (1H, NH, exchangeable with D_2O), 9.68 (1H, CHO), 6.45 (1H, s, *meso*-H), 4.13 (3H, s, >-OCH_3), 3.68 and 3.65 (each 3H, s, $COOCH_3$), 2.90 and 2.85 (each 2H, t, J=7.7 Hz, >-CH_2–), 2.55 and 2.52 (each 2H, t, J=7.7 Hz, -CH_2CO–), 2.31 and 1.91 (each 3H, s, CH_3). Found: *m/z* 402.1777. Calcd for $C_{21}H_{26}N_2O_6$: M, 402.1790.

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