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# Preparation of Mesoverdohemochrome IXα Dimethyl Ester and Mössbauer Spectra of Related Porphyrins

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Mesobiliverdin IXα dimethyl ester (**7**) was obtained by hydrogenation of protobiliverdin IXα dimethyl ester (**8**). Zinc 5-oxamesoporphyrin IX dimethyl ester tetrafluoroborate (**6**) was prepared by the cyclization of **7** with zinc acetate in acetic anhydride followed by a treatment with aqueous sodium tetrafluoroborate. Bis(pyridine) mesoverdohemochrome IXα dimethyl ester (**4**) was obtained by the cyclization of **7** with both iron(II) sulfate and iron(III) chloride in acetic anhydride containing a small amount of pyridine. Bis(tosylmethyl isocyanide) mesoverdohemochrome IXα dimethyl ester (**5**) was prepared by the addition of excess tosylmethyl isocyanide ($TsCH_2NC$) to **4**. Both **4** and **5** reacted with ammonia to give iron(III) 5-azamesoporphyrin IX dimethyl ester (**10**). The Mössbauer spectra of bis(pyridine) verdohemochromes (**4**, **13**, and **14**) showed doublet peaks having parameters of $\delta$=0.41—0.43 mm s$^{-1}$ and $\Delta E_Q$=1.24—1.26 mm s$^{-1}$, indicating that the oxidation states of the central irons of these complexes are iron(II). Bis($TsCH_2NC$) verdohemochromes (**5**, **15**, and **17**) showed single peaks having parameters of $\delta$=0.21—0.28 mm s$^{-1}$. Unstable intermediate and stable final bis($TsCH_2NC$) octaethyl verdohemochromes, obtained by the addition of $TsCH_2NC$ to **4**, were compared with the $^1$H NMR and Mössbauer spectra.

Heme oxygenase catalyzes the oxidative cleavage of the endogenous heme (protoheme IX) to give a blue pigment, biliverdin IXα, in both humans and many animals;[1] the latter is ultimately reduced enzymatically to bilirubin IXα.[2] Verdohemochrome IX (5-oxaprotoporphyriniron(II) complex) was posturated as being an intermediate in the reaction process.[1b,3,4] Verdohemochromes are obtained by a coupled oxidation, termed by Lemberg et al.,[3] of the corresponding hemes with oxygen in the presence of ascorbate in pyridine solution.[5] Because of having no labile vinyl group, mesohemin IX dimethyl ester (**1**) is used as a model compound for the elucidation of the reaction mechanism; a coupled oxidation of **1** with ascorbic acid in aqueous pyridine solution under air gives a mixture of mesoverdohemochromes (α, β, γ, and δ) [bis(pyridine)iron(II) 5-, 10-, 15-, and 20-oxamesoporphyrin complexes]. The mixture gives four mesobiliverdin IX isomers (α, β, γ, and δ)[6] after successive treatments with KOH–MeOH and mineral acid. Many attempts to isolate the four verdohemochrome isomers from the mixture obtained by the coupled oxidation were unsuccessful.

The oxidation state of the central iron of verdohemochromes obtained by a coupled oxidation of the corresponding hemins has been assigned to be iron(II).[7] Jackson et al.[8] obtained mesoverdohemin IXα dimethyl ester (**2**) (iron(III) 15-oxamesoporphyrin IX chloride dimethyl ester) by a reaction of β-hydroxymesohemin IX dimethyl ester (**3**) with oxygen in pyridine. The absorption spectrum of (**2**) was, however, similar to that of a mixture of mesoverdohemochrome IX isomers obtained by a coupled oxidation of mesohemin IX dimethyl ester.[9]

In this paper, we report on the preparation of bis(pyridine)-(**4**), and bis(tosylmethyl isocyanide) mesoverdohemochrome IXα dimethyl ester (**5**). The preparation of zinc 5-oxamesoporphyrin IX dimethyl ester (**6**) is also reported for a comparison of its $^1$H NMR and FAB mass spectra with those of **4** and **5**. Furthermore, the oxidation state of the central iron of mesoverdohemochrome IXα and related porphyrins will be discussed in more detail in terms of Mössbauer, $^1$H NMR, and electronic absorption spectroscopies.

## Results and Discussion

**Preparations of Zinc 5-Oxamesoporphyrin IX Dimethyl Ester (6) and Bis(pyridine)- (4) and Bis(tosylmethyl isocyanide) Mesoverdohemochrome IXα Dimethyl Ester (5).** Metal porphyrins **4**, **5**, and **6** were prepared by a ring closure of mesobiliverdin IXα dimethyl ester (**7**) with corresponding metal ions. Previously, mesobiliverdin IXα was obtained by the oxidation of *d*-urobilin IXα under air[10] and by the oxidation of mesobilirubin IXα with iron(III) chloride.[11] In this study, compound **7** was obtained by the hydrogenation of biliverdin IXα dimethyl ester (**8**) according to a method of Muir and Neuberger;[12] this method was used for the preparation of mesoporphyrin IX from protoporphyrin IX.

Hydrogenation of biliverdin IXα dimethyl ester (**8**) gave compound **7**; the $^1$H NMR spectrum of compound **7** showed the proton signals of two ethyl groups and three *meso*-methine groups (Table 1). The EI mass spectrum of **7** showed a molecular ion peak at *m/z* 614 (base peak). Compound **7** was heated together with $Zn(OAc)_2$ in acetic anhydride at 120 °C under argon, followed by a treatment with an aqueous $NaBF_4$ solution to give zinc 5-oxamesoporphyrin IX dimethyl ester tetrafluoroborate (**6**). The oxamesoporphyrin structure of **6** was supported by the peaks at *m/z* 659 (60.3% of base peak at *m/z* 140), 661 (26.7%), and 663 (24.7%) in FAB mass spectrum, which corre-

Table 1. $^1$H NMR Data ($\delta$ in $CDCl_3$, Multiplicity and $J$/Hz in Parentheses)

| | 7 | 6 | 4 | 5 |
|---|---|---|---|---|
| *meso*-H | 6.70, 5.86, 5.82 | 9.25, 9.00, 8.96 | 8.98, 8.96, 8.64 | 9.04, 9.03, 9.02 |
| $\gg CH_2$ | 2.90(4H, t, 7.3) | 3.80(4H) | 3.84(4H) | 3.80(4H) |
| $COCH_2$- | 2.54(4H, t, 7.3) | 2.94(4H) | 2.86(4H, t, 6.9) | 2.99(4H) |
| -$CH_2CH_3$ | 2.50(2H, q, 7.7) | 3.46(2H, q, 7.7) | 3.54(2H, q, 7.7) | 3.52(2H, q, 7.3) |
| | 2.23(2H, q, 7.7) | 3.35(2H, q, 7.7) | 3.52(2H, q, 7.7) | 3.43(2H, q, 7.3) |
| -$CH_2CH_3$ | 1.20(3H, t, 7.7) | 1.62(3H, t, 7.7) | 1.61(3H, t, 7.7) | 1.66(3H, t, 7.3) |
| | 1.03(3H, t, 7.7) | 1.59(3H, t, 7.7) | 1.60(3H, t, 7.7) | 1.64(3H, t, 7.3) |
| OMe | 3.62, 3.62 | 3.59, 3.58 | 3.45, 3.45 | 3.63, 3.62 |
| $CH_3$ | 2.07, 2.06, 2.06, 1.75 | 3.04, 3.00, 2.99, 2.93 | 3.45, 3.44, 3.44, 3.38 | 3.21, 3.09, 3.06, 3.03 |
| Others | | | 6.44(2H, $\gamma$-HX2 on pyridines) | 7.04(4H, d, 8.1, ortho-H on $TsCH_2NC$) |
| | | | 5.82(4H, $\beta$-HX4 on pyridines) | 6.53(4H, d, 8.1, meta-H on $TsCH_2NC$) |
| | | | 3.22(4H, $\alpha$-HX4 on pyridines) | 2.38(6H, s, $CH_3$X2 on $TsCH_2NC$) |
| | | | | 2.17(4H, s, $CH_2$X2 on $TsCH_2NC$) |

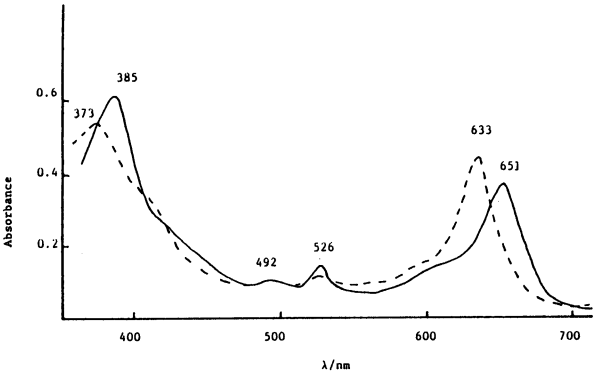



Fig. 1. Absorption spectra ($CH_2Cl_2$) of the (——) bis(pyridine) (**4**) ($1.4\times10^{-5}$ M) and (----) bis($TsCH_2NC$)iron(II) 5-oxamesoporphyrin IX dimethyl ester (**5**).

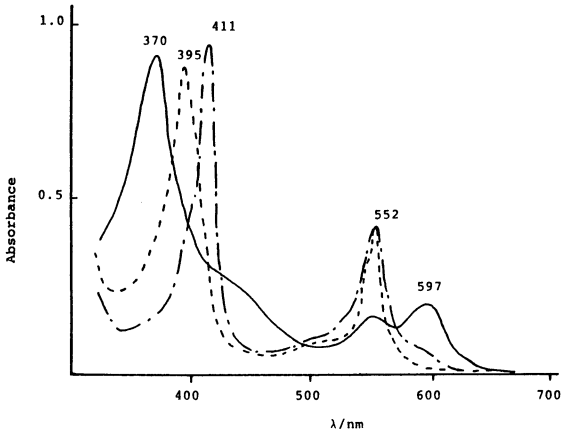



Fig. 2. Absorption spectra (1% pyridine-$CH_2Cl_2$, v/v) of 5-azamesoporphyrin IX iron(III) (**10**) (——) ($2.5\times10^{-5}$ M) and bis(pyridine) (**11**) (----) and bis($TsCH_2NC$)iron(II) 5-azamesoporphyrin IX dimethyl ester (**12**) (-·-·-).

spond to the cations $C_{35}H_{39}N_4O_5{}^{64}Zn^+$, $C_{35}H_{39}N_4O_5{}^{66}Zn^+$, and $C_{35}H_{39}N_4O_5{}^{68}Zn^+$, respectively, and the diamagnetic ring-current shifts of the *meso*-protons ($\delta$ 9.25, 9.00, and 8.96) in its $^1$H NMR spectrum (Table 1). The cyclization of **7** with iron(II) sulfate in acetic anhydride containing a small amount of pyridine at 120 °C under argon, followed by treatment with aqueous $NaBF_4$, gave a green pigment **4**. The FAB mass spectrum of **4** showed a fragment ion peak at $m/z$ 651 (19.2% of the base peak at $m/z$ 140), which corresponds to the unliganded cation $C_{35}H_{39}N_4O_5Fe^+$ of **4**. The absorption spectrum of **4** showed the peaks at 385, 492, 526, and 651 nm (Fig. 1); this spectrum was similar to that of mesoverdohemin IX$\beta$ dimethyl ester (**2**) obtained by Jackson et al. These results indicated that the green pigment was mesoverdohemin IX$\alpha$ dimethyl ester (**9**). However, its $^1$H NMR spectrum was similar to that of the zinc complex **6**, except for the presence of the signals of pyridine ligands; the spectrum showed diamagnetic ring-current shifts of the *meso*-protons ($\delta$ 8.98, 8.96, and 8.64) and the proton signals assigned to the pyridine ligands ($\delta$ 6.44, $\gamma$; 5.82, $\beta$; 3.33, $\alpha$). In analogy with compound **14**,[14] the pyridine complex **4** was easily hydrolyzed to open the tetrapyrrole ring to obtain **7** and was susceptible to oxidation at the *meso*-methine group to give tripyrrins which showed pink spots on TLC (data were not shown). The pyridine ligands of compound **4** easily exchange with tosylmethyl isocyanide ($TsCH_2NC$), which is known to bind to iron(II), but not to iron(III), porophyrins;[13] addition of $TsCH_2NC$ to the solution of **4** resulted in the spectrum of bis($TsCH_2NC$) mesoverdohemochrome IX$\alpha$ dimethyl ester (**5**) with maxima at 373, 492, 526, and 633 nm (Fig. 1). The FAB mass spectrum of **5** showed a base peak at $m/z$ 651 and a fragment ion peak at $m/z$ 1041 (2.7% of the base peak), which correspond to the unliganded cation $C_{35}H_{39}N_4O_5Fe^+$ and to the cation of the bis($TsCH_2$-NC) complex of **5**, respectively. These results suggest that the oxidation state of the central iron of **4** is iron(II), not iron(III). The $^1$H NMR spectrum of **5** showed the diamagnetic ring-current shifts of *meso*-methine protons ($\delta$ 9.04, 9.03,

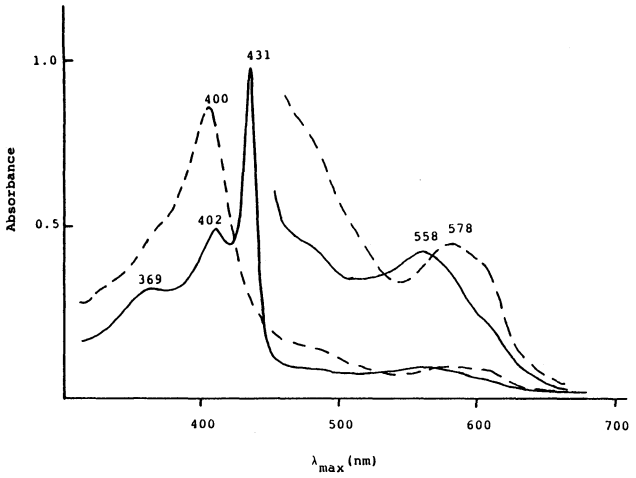



Fig. 3. Absorption spectra ($CH_2Cl_2$) in the reaction of bis($TsCH_2NC$)iron(II) protoporphyrin IX dimethyl ester: —— (16) ($1.8\times10^{-5}$ M); ---- after addition of an excess $K_3[Fe(CN)_6]$ dissolved in 18-crown ether in $CH_2Cl_2$.

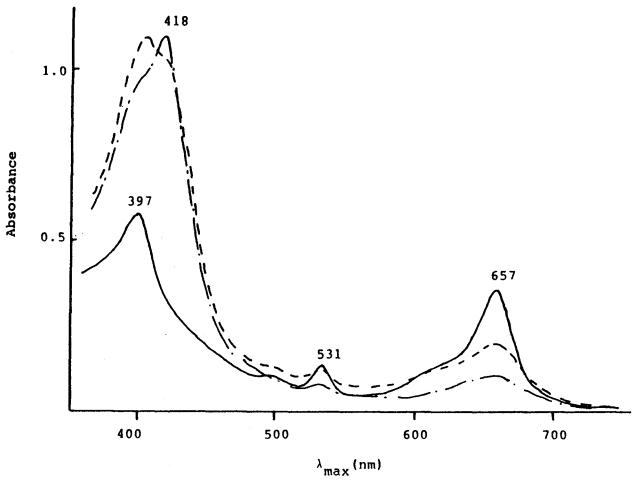
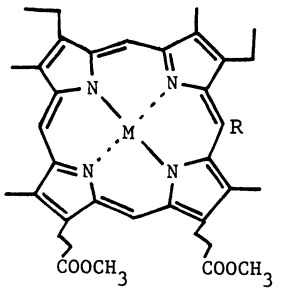
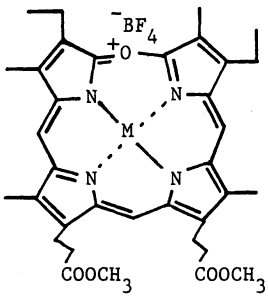



Fig. 4. Absorption spectra (1% pyridine-$CH_2Cl_2$, v/v) in the reaction of bis(pyridine)iron(II) 5-oxaprotoporphyrin IX dimethyl ester (**14**) with $K_3[Fe(CN)_6]$: —— (**14**) ($1.4\times10^{-5}$ M); ---- 1 min after addition of an excess $K_3[Fe(CN)_6]$; -·-·- 10 min after addition of $K_3[Fe(CN)_6]$.




(1) M = Fe(III)·Cl
R = H

(3) M = Fe(III)·Cl
R = OH



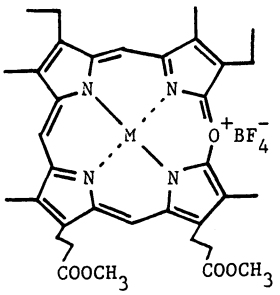

(4) M = Fe(II)·$Py_2$

(5) M = Fe(II)·$(TsCH_2NC)_2$

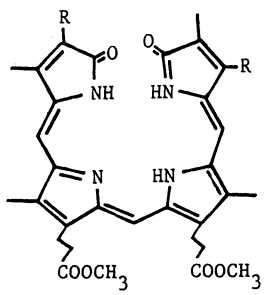
(6) M = Zn

(9) M = Fe(III)·Cl

(20) M = 2H

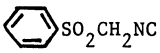



(2) M = Fe(III)·Cl




(7) R = $-CH_2CH_3$

(8) R = $-CH{=}CH_2$

Py = pyridine, $TsCH_2NC$ = $CH_3$  $SO_2CH_2NC$

and 9.02) and of the protons of two $TsCH_2NC$ ligands (Table 1). Both oxaporphyrins **4** and **5** reacted with ammonia to give, after methylation, iron(III) 5-azamesoporphyrin IX dimethyl ester (**10**). The electronic absorption spectrum of compound **10** in 1% pyridine-$CH_2Cl_2$ showed peaks at 370, 553, and 579 nm (Fig. 2). Addition of sodium dithionite to the solution of **10** resulted in the spectrum of bis(pyridine)iron(II) 5-azamesoporphyrin IX dimethyl ester (**11**) with maxima at 395 and 552 nm. Compound **11** gave, upon an addition of excess $TsCH_2NC$, bis($TsCH_2NC$)iron-(II) 5-azamesoporphyrin IX dimethyl ester (**12**), which was identified by FAB mass spectrum data and elemental analysis, as well as the $^1H$ NMR spectral data (Table 1). Thus, both products obtained by the cyclization of mesobiliverdin IX$\alpha$ dimethyl ester (**7**) with iron (II) sulfate and by a coupled oxidation of mesohemin IX dimethyl ester (**1**) showed the same absorption spectra and had the same oxidation state of the central iron.

**Attempts to Obtain Iron(III) 5-Oxaporphyrin Derivatives.** We tried to obtain iron(III) 5-oxamesoporphyrin complex by ring closure of mesobiliverdin IX$\alpha$ dimethyl ester (**7**) with iron(III) chloride instead of iron(II) sulfate; compound **7** was reacted with iron(III) chloride in acetic anhydride containing a small amount of pyridine to give a green pigment. The $^1H$ NMR and absorption spectra of the pigment, however, were the same as those of iron(II) 5-oxamesoporphyrin IX (**4**) obtained by the cyclization of **7** with iron(II) sulfate. Previously, we obtained 5-azamesoporphyrin (**20**) by the treatment of 5-azaprotoporphyrin-iron(III) chloride (**10**) with iron(II) sulfate in acetic acid which contained a small amount of concd HCl; in this reaction, the vinyl groups of **10** was reduced to give **20**. In the cyclization of **7**, iron(III) chloride was dissolved in a small amount of methanol, this mixture then was dissolved in acetic anhydride. Under these conditions, hydrogen might have evolved to reduce iron(III) to iron(II).

It is known that $K_3[Fe(CN)_6]$ oxidizes iron(II) porphyrin to give iron(III) porphyrin. The electronic absorption spectrum of bis($TsCH_2NC$)iron(II) protoporphyrin IX dimethyl ester (**16**) in $CH_2Cl_2$ showed peaks at 369, 406, 431, 558, and 606 nm (Fig. 3). The addition of $K_3[Fe(CN)_6]$ to the solution of **16** resulted in the spectrum of iron(III) protoporphyrin IX complex with maxima at 404, 578, and 602 nm. The absorption spectrum of bis(pyridine)iron(II) 5-oxaprotoporphyrin IX dimethyl ester (**14**) in pyridine showed peaks at 397, 495, 531, and 657 nm (Fig. 4). The addition of $K_3[Fe(CN)_6]$ to the solution of **14** resulted in a new spectrum with maxima at 418 and 658 nm. The large-scaled reaction of **14** with $K_3[Fe(CN)_6]$ was performed, and the mixture was worked-up according to the procedure for **4**. Most of the products of this reaction were tripyrrin derivatives,[14,15] and the starting material was found as a minor component; however, no iron(III) oxaporphyrin complex. Thus, attempts to obtain iron(III) oxaporphyrin were unsuccessful.

**Mössbauer Spectra of Oxa- and Azaporphyriniron Complexes.** Since the reactions of **7** with both iron-(II) sulfate and iron(III) chloride gave the same product, the oxidation state of the central iron of mesoverdohemochrome IX$\alpha$ was reinvestigated by a comparison of the Mössbauer spectra with those of related porphyrins. Mössbauer parameters, isomer shifts ($\delta$) and quadrupole splittings ($\Delta E_Q$), of normal porphyrins and oxa- and azaporphyrins are listed in Tables 2 and 3, respectively. Figures 5a and 5b show typical Mössbauer spectra of bis(pyridine)iron(II)- (**13**) and bis($TsCH_2NC$)iron(II) octaethyl-5-oxaporphyrin (**17**), respectively. The parameters ($\delta$ and $\Delta E_Q$) of **13** and bis(pyridine)iron(II) 5-oxaprotoporphyrin IX dimethyl ester (**14**) were close to those of bis(pyridine)iron-(II) protoporphyrin IX (Fe(pp)$py_2$) and bis(pyridine)-iron(II) tetraphenylporphyrin (Fe(tpp)$py_2$). These results, together with the $^1H$ NMR spectra[9] of **13** and

Table 2. Mössbauer Parameters[16]

i) For low-spin iron(II) porphyrin complexes ($S$=0)

| Compound | Temperature / K | $\delta$[a] / mm s$^{-1}$ | $\Delta E_Q$[b] / mm s$^{-1}$ |
|---|---|---|---|
| Fe(pp)$py_2$[c] | 77 | 0.45 | 1.21 |
| Fe(oep)$py_2$ | 85 | 0.46 | 1.14 |
| Fe(tpp)$py_2$ | 77 | 0.40 | 1.15 |
| ii) For high-spin iron(III) porphyrin complexes ($S$=5/2) | | | |
| Fe(pp)Cl | 4.2 | 0.35 | 0.83 |
| Fe(oep)Cl | 4.2 | 0.41 | 0.93 |
| iii) For diamagnetic carbonyl complexes | | | |
| Fe(tpp)(pip)(CO) | 115 | 0.25 | 0.47 |
| | 295 | 0.18 | 0.53 |
| Fe(pp)(pip)(CO) | 115 | 0.26 | 0.57 |
| | 295 | 0.18 | 0.62 |

a) $\delta$= isomer shift. b) $\Delta E_Q$=quadrupole splitting. c) Porphyrin and ligand abbreviations: pp, protoporphyrin IX; oep, octaethylporphyrin; tpp, tetraphenylporphyrin; py, pyridine; pip, piperidine.

Table 3. Mössbauer Parameters for Oxo- and Azaporphyriniron Complexes and Protoheme Complex at 78 K

| Compound | $\delta$ / mm s$^{-1}$ | $\Delta E_Q$ / mm s$^{-1}$ |
|---|---|---|
| i) Bis(pyridine)iron(II) complexes | | |
| **4** | 0.41 | 1.26 |
| **13** | 0.43 | 1.25 |
| **14** | 0.41 | 1.24 |
| ii) Bis($TsCH_2NC$)iron(II) complexes | | |
| **5** | 0.26 | 0 |
| **17** | 0.28 | 0 |
| **15** | 0.21 | 0 |
| iii) Azaporphyriniron(III) complexes | | |
| **18** | 0.40 | 0.50 |
| **19** | 0.39 | 0.49 |

**14**, were consistent with the idea that the oxidation state of the central iron of compounds **13** and **14** is low-spin iron(II). The parameters ($\delta$ and $\Delta E_Q$) of bis(pyridine)iron(II) 5-oxamesoporphyrin dimethyl ester (**4**) under consideration were also close to those of **13**, **14**, Fe(pp)$py_2$, and Fe(tpp)$py_2$. These results indicate that the product obtained by the cyclization of mesobiliverdin IX$\alpha$ dimethyl ester (**7**) with both iron(II) sulfate and iron(III) chloride, are low-spin iron(II) but not high-spin iron(III), 5-oxamesoporphyrin IX complex.

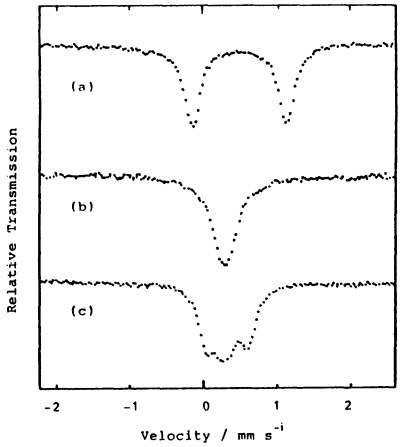
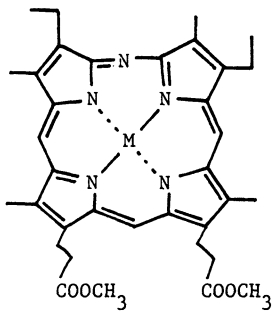



Fig. 5. Mössbauer spectra of bis(pyridine) and bis($TsCH_2NC$)iron(II) octaethyl-5-oxaporphyrins.

The Mössbauer spectra of bis($TsCH_2NC$)iron(II) protoporphyrin IX dimethyl ester (**15**) and bis($TsCH_2$-NC)iron(II) 5-oxaporphyrins (**5**, **16**, and **17**) showed a singlet peak having parameters of $\Delta E_Q$=0 mm s$^{-1}$ and $\delta$=0.21—0.28 mm s$^{-1}$ (Table 3). These parameters differed completely from those of the corresponding bis(pyridine) complexes ($\Delta E_Q$=1.24—1.26 mm s$^{-1}$ and $\delta$=0.41—0.43 mm s$^{-1}$). In general, in going from an Fe(por)$L_2$ complex (por=porphyrinate dianion, L= amine base) to the corresponding Fe(por)L(CO) derivative, $\delta$ decreases by about 0.2 mm s$^{-1}$ and $\Delta E_Q$ by about 0.8—1.0 mm s$^{-1}$. Sams and Tsin[16] explained these phenomena as follows: the quadrupole splitting is small with a decrease in the *d*-electron density on the iron, arising from a strong Fe→CO $\pi$ back bonding; an increase in the CO→Fe $\sigma$ bonding decrease $\delta$. Our experiments on the Mössbauer spectra showed that both parameters ($\delta$ and $\Delta E_Q$) of the bis($TsCH_2NC$) complexes (**5**, **15**, **17**) decreased compared with those of the corresponding bis(pyridine) complexes (**4**, **14**, and **13**); $\delta$ decreases by 0.13—0.22 mm s$^{-1}$ and $\Delta E_Q$ by 1.24—1.26 mm s$^{-1}$. These results seemed to coincide with the explanation for carbon monoxide heme complexes and indicate that bis-

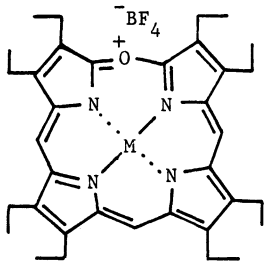



(10) M = Fe(III)·Cl

(11) M = Fe(II)·$Py_2$

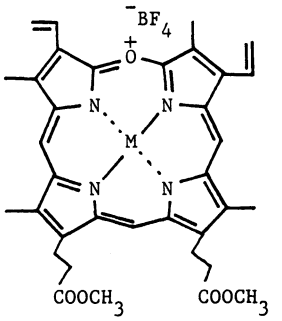
(12) M = Fe(II)·$(TsCH_2NC)_2$

(20) M = 2H



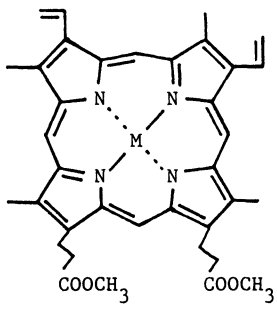

(13) M = Fe(II)·$Py_2$

(17) M = Fe(II)·$(TsCH_2NC)_2$




(14) M = Fe(II)·$Py_2$

(15) M = Fe(II)·$(TsCH_2NC)_2$




(16) M = Fe(II)·$(TsCH_2NC)_2$

(21) M = Fe(III)·Cl

Table 4. Chemical Shifts of Bis($TsCH_2NC$)iron(II) Octaethyl-5-oxaporphyrins[a)]

| Compound | *meso*-H | $-C\underline{H}_2CH_3$ | $-CH_2C\underline{H}_3$ | $CH_3$ | $SO_2CH_2-$ | Aromatic protons |
|---|---|---|---|---|---|---|
| Mixture (**A**+**B**) | 9.15(s) | 3.62—3.40 | 1.76—1.56 | 2.35(s) | 3.03(s) | 7.04(d, 8) |
| | 8.81(s) | | | 2.33(s) | 3.16(s) | 6.50(d, 8) |
| | 9.05(s) | | | | | |
| | 8.89(s) | | | | | |
| **17** | 9.05(s, 2H) | 3.58—3.44 | 1.73—1.57 | 2.33(s, 6H) | 3.16(s, 4H) | 7.03(d, 8) |
| | 8.89(s, 1H) | | | | | 6.54(d, 8) |

a) $\delta$ in $CDCl_3$, multiplicity and $J$/Hz in parentheses.

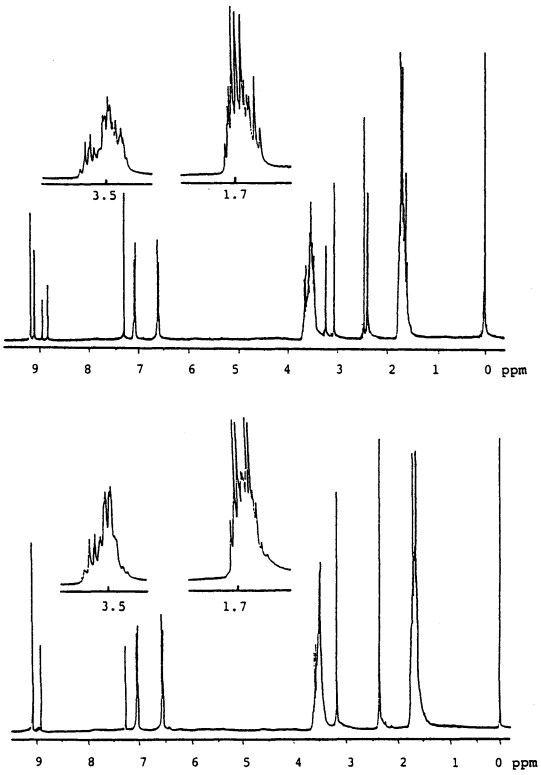


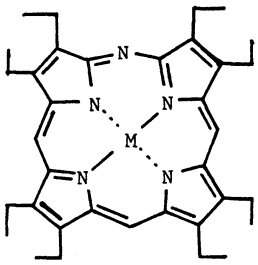

Fig. 6. $^1H$ NMR (270 MHz) spectra of bis($TsCH_2$-NC)iron(II) octaethyl-5-oxaporphyrin complexes.

($TsCH_2NC$) complexes have strong Fe→CN $\pi$ back bonding and Fe←CN $\sigma$ bonding.

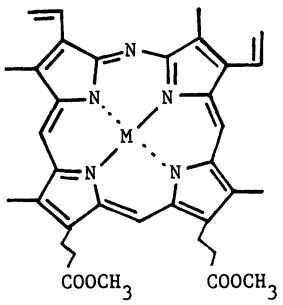
In the course of an investigation of the $^1H$ NMR spectra of bis($TsCH_2NC$)iron(II) octaethyl-5-oxaporphyrin, we found that two kinds of bis($TsCH_2NC$)iron-(II) complexes existed. Bis(pyridine) complex **13** was reacted with excess $TsCH_2NC$, and the reaction mixture was evaporated at lower temperature (<10 °C) according to the method for **4** to give a blue mixture whose $^1H$ NMR spectrum showed two pairs of *meso*-protons due to compounds A and B (ca. 3 : 1, respectively) ($\delta$ 9.15 and 8.81 for compound A, 9.05 and 8.89 for compound B) (Table 4 and Fig. 6). Although compound A was not isolated (because of an easy conversion into compound B), compound B was isolated; in the $^1H$ NMR spectrum, the *meso*-proton at $\delta$ 9.15 and 8.81 for compound A disappeared after the mixture (A+B) was heated at 50 °C in benzene, and the intensity of two peaks at $\delta$ 9.05 and 8.89 of *meso*-protons of compound B increased. The FAB mass spectra of both products (the mixture of A and B and compound B) showed the similar spectra showing a base peak at $m/z$ 591, which corresponds to the cation of $C_{35}H_{43}FeN_4O^+$. Furthermore, the absorption spectra of the mixture and compound B were not appreciably different (see experiment section). These results suggest that both compounds A and B have the same oxaporphyrin ring structure. Compound B was identified with **17** by FAB mass, $^1H$ NMR, and absorption spectra.

The Mössbauer spectrum of compound **17** showed a single peak ($\delta$=0.28 mm $s^{-1}$). On the other hand, the spectrum of the mixture (A and B) showed a triplet peak which consisted of a singlet peak ($\delta$=0.28 mm $s^{-1}$) and a doublet peak ($\delta$=0.31 mm $s^{-1}$ and $\Delta E_Q$=0.55 mm $s^{-1}$) (Fig. 5c). These results suggest that an unstable intermediate (compound A) is first produced by binding $TsCH_2NC$ to iron(II), then the compound A converts to the stable compound B (**17**). The resonating structures of bis($TsCH_2NC$) complex were thought as follows: $Fe{=}C{-}\ddot{N}{-}R \leftrightarrow Fe{-}\bar{C}{\equiv}\overset{+}{N}{-}R$.[17] The Mössbauer parameters for the stable compound B indicate that the electronic structure of the bonding between iron(II) and isocyanide ligand can be assigned as a double-bonded structure, due to the strong Fe→CN $\pi$ and Fe←CN $\sigma$ bonding. On the other hand, the fact that the Mössbauer parameters of the unstable compound A are larger than those of the stable compound B suggests a decrease in the Fe→CN $\pi$ back donation for compound A. Though the electronic structure of the bonding between iron(II) and isocyanide ligands for the unstable intermediate A has not yet been confirmed, we may presume that $TsCH_2NC$ binds to iron-(II) to initially form an unstable bonding, such as Fe←CN $\sigma$ or $Fe{\leftarrow}\overset{C}{\underset{N}{|||}}$ bonding. In this case the bonding is formed between the $\pi$ electrons of CN group and Fe; these unstable structures then convert to the stable double-bonded structure, resulting in the formation of compound B.

The Mössbauer spectra of (octaethyl-5-azaporphyrinato)iron(III) chloride (**18**) and 5-azaprotoporphyrin IX iron(III) chloride dimethyl ester (**19**) were obtained at 78 K. The parameters of the spectra are listed in



(18) M = Fe(III)·Cl



(19) M = Fe(III)·Cl

Table 3-iii. It is generally said that because of a second-order Doppler shift, the isomer shift decreases with an increase in the temperature, while the quadrupole splitting does not appreciably change upon a variation of the temperature in complexes of this type. From this suggestion, our experiments showed that the $\delta$ values of **18** and **19** were larger than that of Fe(III)(oep)Cl, and $\Delta E_Q$ values of **18** and **19** were smaller than that of Fe(III)(oep)Cl.[18] These effects seem to be due to the substitution of a nitrogen atom for a carbon atom of the porphyrin moiety. These effects will be discussed later in further detail.

## Experimental

**Materials.** Protohemin IX dimethyl ester was prepared by methylation of protohemin IX, purchased from Aldrich. Biliverdin IX$\alpha$ was prepared by oxidation of bilirubin IX$\alpha$, purchased from Aldrich, with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone. Bis(pyridine) octaethylverdohemochrome was prepared according to a published procedure. Tosylmethyl isocyanide ($TsCH_2NC$), purchased from Aldrich, was recrystallized from ethanol after treatment with active carbon in ethanol. The solvents for analyses were of analytical grade. All other solvents and chemicals were of reagent grade. Analytical thin-layer chromatography (TLC) was performed on DC-Alufolien Kieselgel 60 HF254 (Merck). Wacogel C-200 was used for column chromatograph.

**Measurements.** Melting points were determined with a Yanoko micro melting-point apparatus. Electronic absorption spectra were recorded with a Hitach U-3200 Spectrophotometer. EI and FAB mass spectra were obtained with a JEOL JMS 300 mass spectrometer. $^1H$ NMR spectra at 270 MHz of samples in $CDCl_3$ solution containing $Me_4Si$ were recorded with a JEOL JNM-GX 270 FT NMR spectormeter. The Mössbauer spectra were recorded with an Austin Science Associated Mössbauer Spectrometer Controller. A 20 mCi single-line $^{57}Co$ source in Rh matrix was used. The velocity scale of the spectrometer was calibrated by using the spectra of the metallic iron. Isomer shifts are reported relative to metallic iron at room temperature. By fitting the resonance line-shape to a Lorentzian function, the obtained Mössbauer parameters are within $\pm 0.002$ mm s$^{-1}$ for isomer shifts and $\pm 0.005$ mm s$^{-1}$ for quadrupole splittings. Low-temperature measurements were performed using a CA thermocouple embedded in a copper sample holder with an Air Products and Chemicals Model LT-3-110 Liquid Transter Helitran. The precision of the temperature measurement was with approximately $\pm 0.1$ K over a period of several hours.

**Preparation of Mesobiliverdin IX$\alpha$ Dimethyl Ester (7).** In 50 ml of anhydrous formic acid, methyl methacrylate (400 mg) was dissolved, and 10% Pd-charcoal (75 mg) was added. The mixture was added with biliverdin IX$\alpha$ dimethyl ester (**8**) (350 mg) and passed with $H_2$ at 50 °C for 50 min. The mixture was filtered. The filtrate was diluted with $H_2O$ (50 ml) and extracted with $CH_2Cl_2$ (50 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 was subjected to column chromatography (benzene-acetone, gradient up to 10%) to isolate mesobiliverdin IX$\alpha$ dimethyl ester (**7**) (220 mg, 62.5%). EI MS *m/z* (rel intensity) 614 ($M^+$, 100), 598 (19.5), 583 (23.8), 555 (26.2), 541 (47.6), 527 (17.1), 467 (85.7), 454 (47.6). Found: C, 68.30; H, 6.89; N, 9.03%. Calcd for $C_{35}H_{42}N_4O_6$: C, 68.38; H, 6.89; N, 9.11%.

**Zinc 5-Oxamesoporphyrin IX Dimethyl Ester Tetrafluoroborate (6).** To a solution of mesobiliverdin IX$\alpha$ dimethyl ester (**7**) (150 mg), a solution of zinc acetate dihydrate (185 mg) in MeOH (5 ml) was added. The mixture was warmed at 60 °C for 30 min and filtered. The filtrate was evaporated and the residue was dissolved in $CH_2Cl_2$ (5 ml) and acetic anhydride (30 ml). After the mixture had been heated at 120 °C for 30 min under argon, the solvent was removed with a vigorous stream of argon at 140 °C. The resulting residue was chromatographed on silica gel with $CH_2Cl_2$-MeOH (gradient up to 5%) to give the major green pigment. The pigment was dissolved in $CH_2Cl_2$, washed with saturated aqueous $NaBF_4$ and water, dried, and evaporated to give a residue, which was subjected to column chromatography to isolate compound **6** (125.2 mg, 68.5%), mp 300 °C. Found: C, 56.03; H, 5.29; N, 7.35%. Calcd for $C_{35}H_{39}N_4O_5ZnBF_4$: C, 56.21; H, 5.26; N, 7.49%. FAB MS *m/z* (rel intensity) 659 (60.3), 661 (26.7), 663 (24.7), 406 (40.0), 252 (38.5), 140 (100).

**Cyclization of 7 with Iron(III) Sulfate.** To a solution of compound **7** (110 mg) in acetic anhydride (50 ml) and pyridine (5 ml), a solution of iron(II) sulfate heptahydrate (100 mg) in methanol (7 ml) was added under argon. The reaction mixture was heated for 30 min at 120 °C under argon, and the solvent was removed by a vigorous flow of argon at 140 °C to give a residue. The residue was extracted with 2% pyridine-$CH_2Cl_2$ (50 ml×3) and the combined organic extracts were washed succesively with saturated aqueous $NaBF_4$ and water, dried, and evaporated to give a green residue. The residue was chromatographed on silica gel with $CH_2Cl_2$ containing 1% pyridine-MeOH (gradient up to 5%) to give bis(pyridine)iron(II) mesoverdohemochrome IX$\alpha$ dimethyl ester (**4**) (94.1 mg, 58.6%). FAB MS *m/z* (rel intensity) 651 (17.8), 252 (56.2), 235 (28.8), 140 (100). Calcd

for $C_{45}H_{49}N_6O_5FeBF_4$: C, 60.28; H, 5.51; N, 9.37%. Found: C, 59.90; H, 5.60; N, 9.22%.

**Cyclization of 7 with Iron(III) Chloride.** To a solution of **7** (50 mg) in acetic anhydride (50 ml) and pyridine (5 ml), a solution of iron(III) chloride monohydrate (100 mg) in methanol (3 ml) was added. The reaction mixture was treated and worked-up according to the procedure just described above for **4**. The resulting green pigment (36.3 mg, 47.7%) was identified with **4** by TLC and $^1H$ NMR spectroscopy.

**Bis(TsCH$_2$NC)iron(II) Complex (5).** Compound **4** (102 mg) was dissolved together with TsCH$_2$NC (350 mg) in $CH_2Cl_2$ (5 ml). The solution was subjected to column chromatography ($CH_2Cl_2$-MeOH, gradient up to 5%) to give compound **5** (98.4 mg, 76.6%), mp>300 °C (after recrystallization from benzene-peteroleum ether). Found: C, 56.11; H, 5.12; N, 7.15%. Calcd for $C_{53}H_{57}N_6O_9S_2FeBF_4$: C, 56.39; H, 5.09; N, 7.44%. FAB MS *m/z* (rel intensity) 1041 (2.7, $M^+-BF_4$), 651 (100, $M^+-2\times TsCH_2NC-BF_4$).

**Iron(III) 5-Azamesoporphyrin IX Dimethyl Ester (10).** a) To a solution of compound **4** (53 mg) in pyridine (15 ml) and concd aqueous ammonia (10 ml) was added. The reaction mixture was stirred overnight at room temperature. The mixture was diluted with water (50 ml) and extracted with $CH_2Cl_2$ (50 ml×3). The combined organic extracts were washed with saturated aqueous NaCl and water, dried, and evaporated to give a residue. The residue was dissolved in 5% $H_2SO_4$-MeOH (100 ml) and allowed to stand overnight at room temperature. The reaction mixture was extracted with $CH_2Cl_2$ (100 ml×3). The extract was worked-up in the usual way. After recrystallization from $CH_2Cl_2$-peteroleum ether, compound **10** (32 mg, 39.5%) was obtained, mp>300 °C. Found: C, 61.43; H, 5.44; N, 9.93%. Calcd for $C_{35}H_{39}N_5O_4$-FeCl: C, 61.37; H, 5.34; N, 10.22%. b) To a solution of **5** (30 mg) in pyridine (1 ml) and concd aqueous ammonia (10 ml) was added. The reaction mixture was stirred overnight at room temperature. The reaction mixture was worked-up according to the procedure of a). The compound **10** (6 mg, 33%) was obtained.

**Bis(TsCH$_2$NC)iron(II) Complex (12).** The solution of compound **10** (100 mg) in $CH_2Cl_2$ (50 ml) containing pyridine (5 ml) was washed with aqueous $Na_2S_2O_4$ and water. The organic solution was added with TsCH$_2$NC (30 mg) and subjected to column chromatography ($CH_2Cl_2$-acetone, gradient up to 5%) to give **12** (103 mg, 67.8%). FAB MS *m/z* 649 (base peak, $M^+-2\times TsCH_2NC$) Found: C, 61.17; H, 5.53; N, 9.40%. Calcd for $C_{53}H_{57}N_7O_8S_2Fe$: C, 61.21; H, 5.52; N, 9.43%.

**5-Azamesoporphyrin IX Dimethyl Ester (20).** To a solution of compound **12** (40 mg) in pyridine (0.5 ml) and acetic acid (15 ml), a solution of iron(II) sulfate heptahydrate (40 mg) in concd HCl (1 ml) was added under argon, and the reaction mixture was poured into ice water (30 ml) and extracted with $CH_2Cl_2$ (25 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. The residue was dissolved in 5% $H_2SO_4$-MeOH (50 ml) and allowed to stand overnight. The mixture was poured into ice water (100 ml) and extracted with $CH_2Cl_2$ (50 ml×3). The combined organic extracts were washed successively with saturated aqueous sodium hydrogencarbonate and water, dried over $Na_2SO_4$, and filtered. The filtrate was evaporated to give a residue which was subjected to column chromatography ($CH_2Cl_2$) to obtain azaporphyrin (**20**) (15 mg, 52.4%). Compound **20** was identified with authentic sample[14] by TLC, $^1H$ NMR and mass spectra.

**Bis(TsCH$_2$NC)iron(II) Protoporphyrin IX Dimethyl Ester (16).** To a solution of protohemin IX dimethyl ester (**21**) (100 mg) in pyridine (10 ml), a solution of $Na_2S_2O_4$ (50 mg) in water (1 ml) was added. The mixture was poured into ice water (50 ml) and extracted with $CH_2Cl_2$ (50 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. The residue was dissolved in $CH_2Cl_2$ (2 ml) and was added with TsCH$_2$NC (80 mg). The mixture was subjected to column chromatography ($CH_2Cl_2$-MeOH, gradient up to 5%) to obtain compound **16** (115.4 mg, 75.8%). Compound **16** was recrystallized from benzene-light peteroleum ether (1 : 1). Found: C, 62.63; H, 5.26; N, 8.07%. Calcd for $C_{54}H_{54}N_6O_8S_2Fe$: C, 62.66; H, 5.26; N, 8.12%. $^1H$ NMR ($CDCl_3$) $\delta$=9.77, 9.73, 9.66, and 9.55 (each, 1H, s, *meso*-H), 8.25 (2H, dd, $J$=17.6, 11.7 Hz, $H_C$ and $H_F$), 6.92 (4H, broad s, *ortho*-protons on TsCH$_2$NC×2), 6.23 (2H, d, $J$=17.8 Hz, $H_A$ and $H_D$), 6.00 (2H, dd, $J$=11.7, 1.5 Hz, $H_B$ and $H_E$), 5.69 (4H, broad s, *meta*-protons on TsCH$_2$NC×2), 4.27 (4H, t, $J$=7.2 Hz, -CH$_2$-×2), 3.63, 3.60, 3.54, and 3.52 (each 3H, s, $CH_3$), 3.22 (4H, t, $J$=7.2 Hz, -CH$_2$CO-×2), 2.40 (6H, s, $CH_3$×2 on TsCH$_2$NC×2), 2.23 (4H, s, $CH_2$×2 on TsCH$_2$NC×2).

**Bis(TsCH$_2$NC)iron(II) Octaethyl-5-oxaporphyrin (17).** Bis(pyridine)iron(II) octaethyl-5-oxaporphyrin (**13**, 50 mg) was dissolved in $CH_2Cl_2$ (2 ml) and added with TsCH$_2$NC (50 mg). The mixture was subjected to column chromatography ($CH_2Cl_2$-acetone, gradient up to 10%). The blue coloured eluant was evaporated at below 10 °C to give a blue oil (48.4 mg, 76%) which showed one spot on TLC ($R_f$ 0.37; benzene-acetone 85 : 15). UV $\lambda_{max}$ ($CH_2Cl_2$) nm ($\varepsilon_{mM}$) 628 (45.2), 583sh (13.1), 529 (11.5), 494 (10.5), 402sh (35.5), 372 (54.5). FAB MS *m/z* 591 ($M^+-2\times TsCH_2NC-BF_4$, 100%). IR ($CHCl_3$) 2960, 2930, 2860. 2140, 1708, 1610, 1595, 1560, 1450, 1400, 1380, 1340, 1260, 1145, 1095, 1060, 1005, 980, 960 cm$^{-1}$. The blue oil was dissolved in benzene and warmed at 50 °C for 30 min. The solution was evaporated to give a residue which showed the same $R_f$ value and FAB mass spectrum as those of the blue oil obtained at the lower temperature. UV $\lambda_{max}$ ($CH_2Cl_2$) nm ($\varepsilon_{mM}$) 627 (46.3), 582sh (13.0), 530 (12.4), 496 (12.1), 404 (37.0), 372 (56.3). IR ($CHCl_3$) 3080, 3000, 2960, 2950, 2860, 2140, 1960, 1817, 1605, 1595, 1480, 1380, 1340, 1263, 1150, 1480, 1060, 1030, 980, 960 cm$^{-1}$.

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## References

1) R. Schmid and A. F. McDonaph, "The porphyrins," ed by D. Dolphin, Academic press, New York (1979), Vol. VI, p. 257; E. C. Foulkes, R. Lemberg, and P. Purdom, *Proc. Roy. Soc. London Ser. B*, **138**, 368 (1951); R. Lemberg, B. Cortis-Jones and, M. Norrie, *Biochem. J.*, **32**, 149 (1938).

2) R. Tenhunen, H. S. Marver, N. R. Pimstone, W. F. Trager, D. Y. Cooper, and R. Schmid, *Biochemistry*, **11**, 1716 (1972).

3) R. Lemberg, B. Cortis-Jones, and M. Norrie, *Bio-*

*chem. J.*, **32**, 149 (1938).

4) S. Saito and H. A. Itano, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1393 (1982).

5) R. Lemberg, *Biochem. J.*, **29**, 2322 (1935); E. Y. Levin, *Biochemistry*, **5**, 2845 (1966).

6) R. Lemberg, *Rev. Pure Appl. Chem.*, **6**, 1 (1956); P. O'Carra, "Porphyrins and Metalloporphyrins," ed by K. M. Smith, Elsevier Scientific Publishing Company, Amsterdam (1975), p. 123; R. Schmid and A. F. McDonaph, *Ann. N. Y. Acad. Sci.*, **244**, 533 (1975).

7) J. C. Lagarias, *Biochem. Biophys. Acta*, **717**, 12 (1982); T. Hirota and H. A. Itano, *Tetrahedron Lett.*, **24**, 995 (1983); S. Sano, Y. Sugiura, Y. Maeda, S. Ogawa, and I. Morishima, *J. Am. Chem. Soc.*, **103**, 2888 (1981).

8) A. H. Jackson, G. W. Kenner, and K. M. Smith, *J. Chem. Soc. C*, **1968**, 302.

9) S. Saito and H. A. Itano, *J. Chem. Soc., Perkin Trans. 1*, **1986**, 1.

10) C. J. Watoson, *J. Clin. Pathol.*, **16**, 1 (1963).

11) H. Fischer, H. Baumgarter, and R. Hess, *Z. Physiol. Chem.*, **206**, 201 (1932).

12) H. Muir and A. Neuberger, *Biochem. J.*, **45**, 163 (1949).

13) L. Pauling, "Haemoglobin," ed. F. J. W. Roughton and J. C. Kendrew, Butterworths Scientific Publications, London (1949), p. 57.

14) S. Saito and N. Tamura, *Bull. Chem. Soc. Jpn.*, **60**, 4037 (1987).

15) S. Saito and H. A. Itano, *J. Chem. Soc., Perkin Trans. 1*, **1987**, 1183.

16) J. R. Sams and T. B. Tsin, "The Porphyrins," ed by D. Dolphin, Academic press, New York (1979), Vol. IV, p. 425.

17) L. Pauling, *Stanford Medical Bull.*, **6**, 215 (1948).

18) B. W. Fitzsimmons, J. R. Sams, and T. B. Tsin, *Chem. Phys. Lett.*, **38**, 588 (1976).