Partial Syntheses of the Isomerically Pure Magnesium(II) Protoporphyrin IX Monomethyl Esters, and Their Identification

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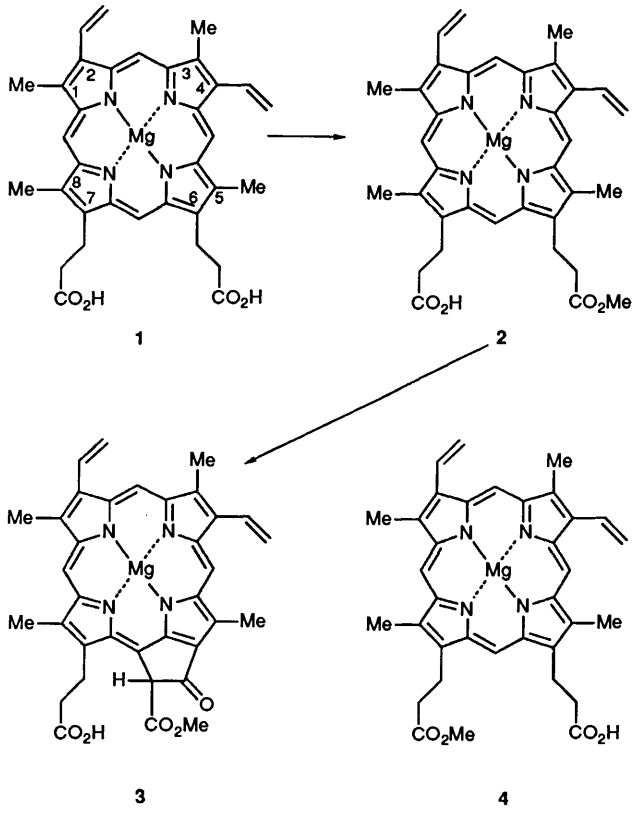
Treatment of 2,4-diacetyldeuteroporphyrin IX with oxalyl dichloride and t-butyl alcohol gives mainly the two isomeric mono-t-butyl esters, **10** and **11**, which can be separated by thick-layer chromatography on silica gel. These monoesters can be individually transformed, *via* a sequence of steps, into the corresponding isomerically pure protoporphyrin IX monomethyl esters, **5** and **6**, one being the magnesium(II)-free derivative of a key intermediate in the biosynthetic pathway to chlorophylls and bacteriochlorophylls. The isomerically pure monomethyl esters **5** and **6** were magnesiated and the resulting magnesium(II) protoporphyrin IX monomethyl esters **2** and **4**, respectively, were challenged with a suspension of isolated developing chloroplasts in the presence and absence of added *S*-adenosylmethionine. The isomerically pure isomer which was converted into magnesium(II) 2,4-divinylpheoporphyrin a_5 **3** in the absence of the SAM cofactor was identified as the physiologically significant 6-methyl ester **2**.

During chlorophyll *a* biosynthesis, magnesium(II) protoporphyrin IX **1** is converted into magnesium(II) protoporphyrin IX monomethyl ester **2**, which undergoes oxidative cyclization to afford magnesium(II) 2,4-divinylpheoporphyrin a_5, **3**, which we shall refer to throughout as 'divinylprotochlorophyllide'.[1,2] The intermediate **2** is esterified uniquely at the 6-propionate, and the 7-propionic ester **4** is said to be inert, except for the presence of esterase(s),[3,4] which convert the 7-propionic ester **4** back into the free dicarboxylic acid **1** whereupon *S*-adenosylmethionine magnesium protoporphyrin IX methyltransferase regenerates the 6-propionic ester **2**. Since in chloroplasts *S*-adenosylmethionine (SAM) is limiting, in the absence of added SAM the 7-propionic ester **4** should not be significantly converted into divinylprotochlorophyllide **3**. On the other hand, the 6-propionic ester **2** should be transformed into keto diester **3** in good yield in the absence of SAM. Long incubations should highlight a stimulation due to exogenous SAM, even when the physiological 6-propionic ester **2** is used as the substrate.

This paper describes an efficient route for the partial synthesis of haem derivatives of the two pure isomers **2** and **4**, and definitive structure identification using incubations with an isolated chloroplast preparation.

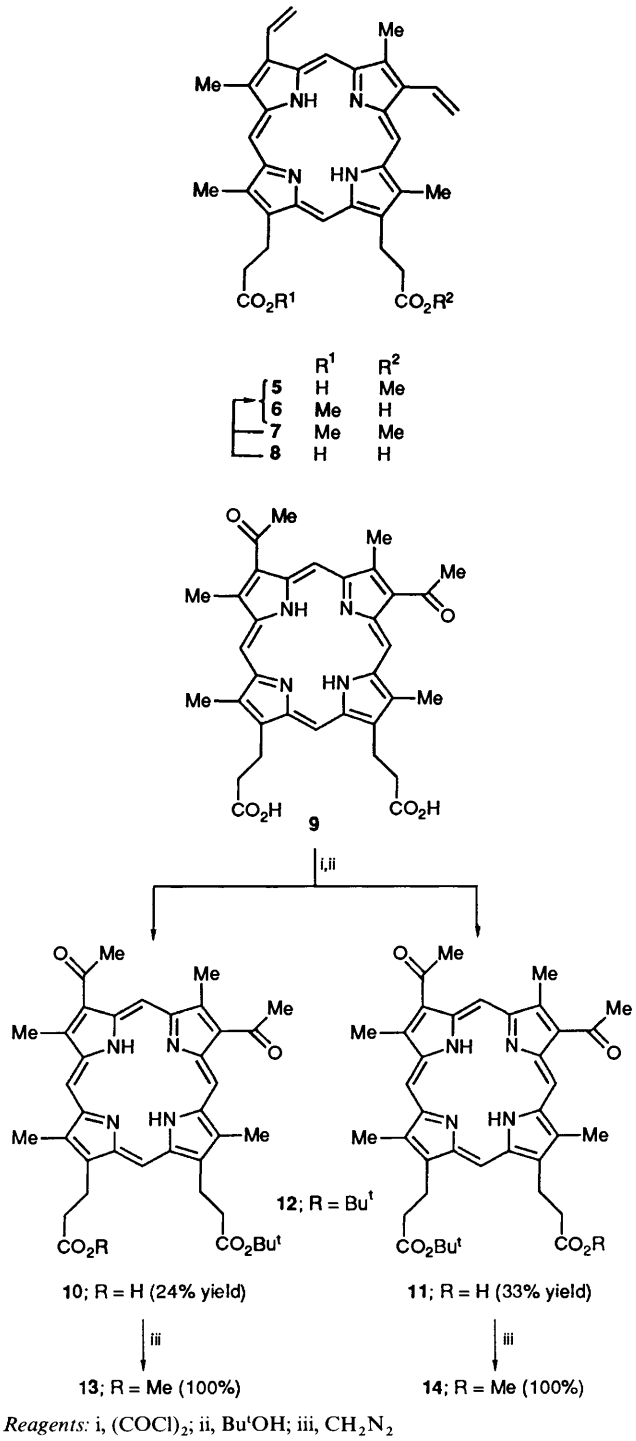
Synthesis of the Isomerically Pure Magnesium(II) Monomethyl Esters **2** *and* **4**.—The isomeric mixture of protoporphyrin IX monomethyl esters **5** and **6** can be readily prepared by partial hydrolysis[5] of the dimethyl ester **7** or by partial esterification[6] of protoporphyrin IX **8** followed by separation of the isomeric mixture **5** and **6** from residual substrates **7** and **8**. In our hands, separation of the mixture **5**/**6** has been very difficult, if not impossible, even using careful high-performance liquid chromatography (HPLC). However, in other work we have noted that acetylporphyrin isomers are readily separable, even on the 5-gram scale.[7] We therefore chose to investigate separation of 2,4-diacetyldeuteroporphyrin isomers.

2,4-Diacetyldeuteroporphyrin IX **9** was treated with oxalyl dichloride and t-butyl alcohol to give substantially the t-butyl esters **10** and **11** (contaminated with a little of the free acid **9** and the di-t-butyl ester **12**). These were separated from starting material **9** and diacetyldeuteroporphyrin IX di-t-butyl ester **12** by using column chromatography on silica gel. The two isomers **10** and **11** were readily separated by use of thick-layer chromatography on silica gel to give a fast running Band 'A' [**10** (24% yield from **9**)] and a slower running Band 'B' [**11** (33%)].†

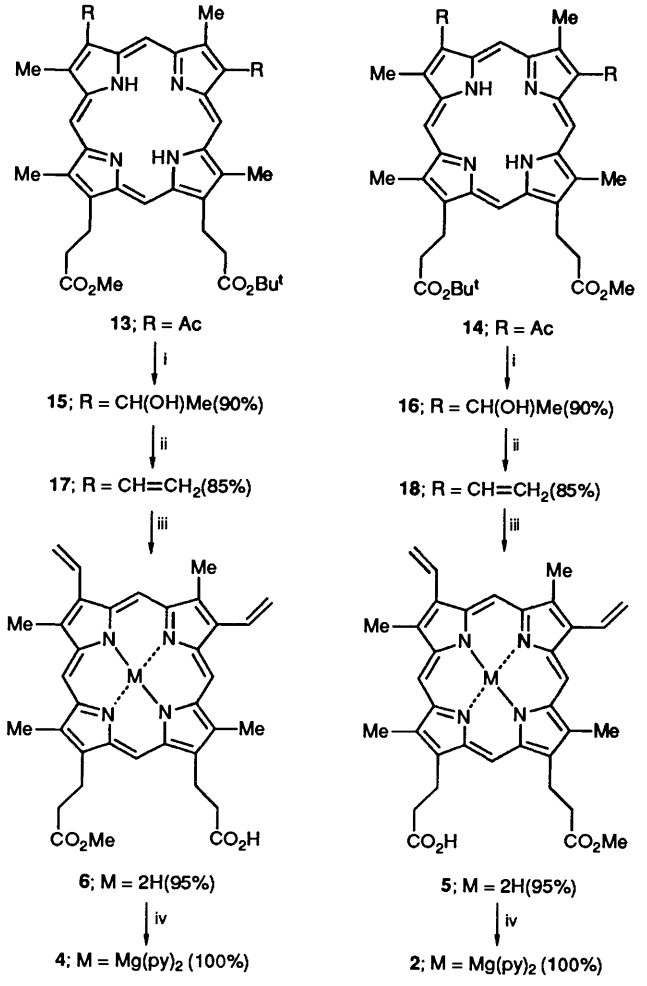


Treatment of the individual isomers with diazomethane gave a quantitative yield of the mixed esters **13** and **14**. These were converted into the individual haematoporphyrin IX mixed esters **15** and **16** (90% yield) by reduction with sodium borohydride, and from there into the protoporphyrin IX analogues **17** and **18** (85% yield), respectively, by dehydration with toluene-*p*-sulphonic acid (PTSA) in hot *o*-dichlorobenzene.

† At this point, of course, we were unaware of the isomeric identities of compounds **10** and **11**, but see later.



Reagents: i, $(COCl)_2$; ii, Bu^{t}OH; iii, CH_2N_2



Reagents and conditions: i, $NaBH_4$, MeOH, 0 °C; ii, PTSA, reflux; iii, TFA; iv, $Mg(ClO_4)_2$, pyridine

Table 1 Identification of isomers **2** and **4** by means of the biological cyclization reaction to give divinylprotochlorophyllide

Porphyrin substrate derived from	Time of incubation (*t*/min)	Divinylprotochlorophyllide synthesis (in pmol h^{-1})	
		(a) − SAM	(b) + SAM
Band 'A' (*i.e.*, **4**)	20	36.6	83.7
Band 'A' (*i.e.*, **4**)	60	42.8	298.4
Band 'B' (*i.e.*, **2**)	20	129.8	118.5
Band 'B' (*i.e.*, **2**)	60	386.7	408.6

All samples contained 0.8 mol dm^{-3} NADPH (as regenerating system), 10 μmol dm^{-3} porphyrin substrate, and plastid protein (2.6 mg). 1 mmol dm^{-3} SAM was added to samples in column (b) but not in column (a). The no-substrate control contained divinylprotochlorophyllide (21 pmol) that was subtracted from the tabulated values.

Brief treatment with trifluoroacetic acid (TFA) gave the protoporphyrin IX monomethyl esters **6** and **5** in 85% yield; these were magnesiated with magnesium(II) perchlorate in hot pyridine to provide quantitative yields of the chelates **4** and **2** respectively.

Identification of the Isomers **2** *and* **4**.—Compound **4**, obtained from Band 'A',[6] was barely converted at all into the divinylprotochlorophyllide **3** by a developing chloroplast suspension in the absence of the biological methylating agent SAM (Table 1; see Fig. 2). However, the conversion of compound **4** into compound **3** was greatly stimulated by adding SAM. On the other hand, the conversion of compound **2** (obtained from Band 'B') remained relatively unaffected up to 40 min (Table 1; Fig. 1), suggesting that the latter (*i.e.*, compound **2**) is the physiological 6-propionic ester isomer. According to our previous results,[1,2] the cyclization of the physiological isomer should also become stimulated by SAM during longer incubations, as the methyl ester functionality is hydrolysed and needs to be regenerated before the substrate can be cyclized. This was observed experimentally (Fig. 1). Even in the presence of added SAM the rate of cyclization of compound **4** remained considerably lower than the rate of cyclization of compound **2** (compare Fig. 2 with Fig. 1), confirming the identity of compound **4** as the non-physiological 7-propionic methyl ester.

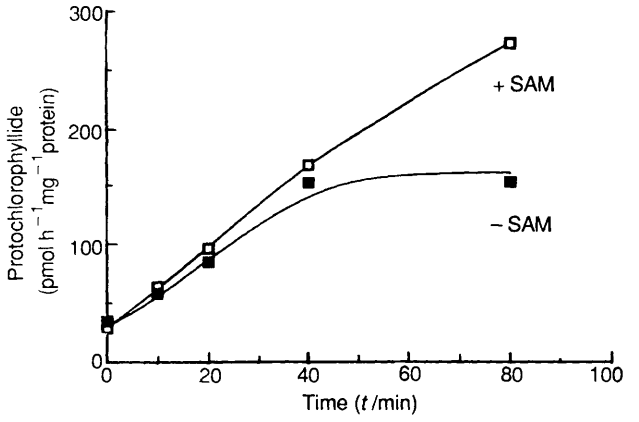


Fig. 1 Time-course of the cyclization of the putative physiological magnesium(II) protoporphyrin IX monomethyl ester isomer **2** to give divinylprotochlorophyllide **3** in the presence and absence of *S*-adenosyl methionine (SAM)

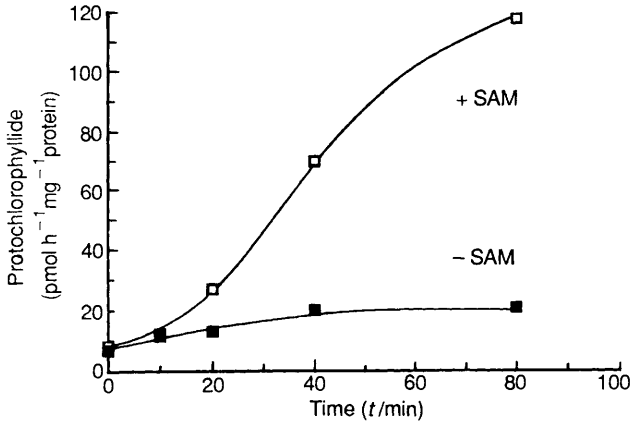


Fig. 2 Time-course of the cyclization of the putative physiological magnesium(II) protoporphyrin IX monomethyl ester isomer **4** to give divinylprotochlorophyllide **3** in the presence and absence of *S*-adenosyl methionine (SAM)

Experimental

M.p.s were measured on a hot-stage apparatus and are uncorrected. Silica gel 60 (70–230 mesh, Merck) or neutral alumina (Merck; usually Brockmann Grade III, *i.e.* deactivated with 6% water) were used for column chromatography. Preparative TLC (PLC) was carried out on 20 × 20 cm glass plates coated with Merck G 254 silica gel (1 mm thick). Analytical TLC was performed using Merck F254 silica gel (precoated sheets, 0.2 mm thick). Reactions were monitored by TLC and spectrophotometry and were carried out under nitrogen and in the dark. ^{1}H NMR spectra were obtained in deuteriochloroform solution at 300 MHz, using a General Electric QE300 spectrometer; chemical shifts are expressed in ppm relative to chloroform (δ_H 7.258). Elemental analyses were performed at Midwest Microlab., Ltd, and were carried out on mixtures of the isomeric pairs obtained from a separate run through the synthesis in which the isomer separation was omitted. Electronic absorption spectra were measured in dichloromethane solution, using a Hewlett-Packard 8450A spectrophotometer.

Organic Synthesis

2,4-*Diacetyl*-6-(2-*t*-*butoxycarbonylethyl*)-7-(2-*carboxyethyl*)-1,3,5,8-*tetramethylporphyrin* **10** *and* 2,4-*Diacetyl*-7-(2-*t*-*butoxycarbonylethyl*)-6-(2-*carboxyethyl*)-1,3,5,8-*tetramethylporphyrin* **11**.—2,4-Diacetyldeuteroporphyrin IX **9** (185 mg) (obtained by hydrolysis of the corresponding dimethyl ether[7]) in dry dichloromethane (10 cm^3) was refluxed under N_2 for 10 min and the solution was then cooled to room temperature. Oxalyl dichloride (2 cm^3) was added at once *via* a syringe and the solution was then refluxed at 45 °C under N_2 for 30 min, then was cooled to room temperature, the solvent and excess of oxalyl dichloride were removed under reduced pressure, and the residue was dissolved in dichloromethane (10 cm^3) and the solution was evaporated to dryness under reduced pressure. The residue was redissolved in a mixture of dry dichloromethane (35 cm^3) and t-butyl alcohol (3 cm^3) and the reaction mixture was stirred at 45 °C under N_2 for 10 min before being poured into water (250 cm^3) and extracted with dichloromethane (200 cm^3). The organic phase was washed with water (3 × 150 cm^3) and after removal of the solvent the residue was dissolved in 10% methanol–dichloromethane and chromatographed on a silica gel column, with 10% MeOH–CH_2Cl_2 as eluant. The major fraction was collected, evaporated to dryness, and the residue was then dissolved in tetrahydrofuran (THF) (15 cm^3) and diluted with CH_2Cl_2 (200 cm^3). This mixture was washed with water (7 × 150 cm^3) to remove a small amount of silica gel; the organic phase was dried over Na_2SO_4, the solvent was removed, and the residue was crystallized from THF–n-hexane to give an isomeric mixture of monoesters **10** and **11** (115 mg, 57%). After brief treatment with excess of ethereal diazomethane, the mixture of diacetyldeuteroporphyrin IX monomethyl mono-t-butyl esters **13**/**14** was obtained; δ_H($CDCl_3$) 10.31 (1 H, s, meso H), 10.29 (1 H, s, meso H), 10.18 (1 H, s, meso H), 10.16 (1 H, s, meso H), 9.46 (2 H, s, meso H), 9.14 (1 H, s, meso H), 9.12 (1 H, s, meso H), 4.20–4.11 (8 H, m, CH_2CH_2CO), 3.67 (3 H, s, OMe), 3.65 (3 H, s, OMe), 3.56 (6 H, s, 2 × ring Me), 3.50 (3 H, s, ring Me), 3.47 (3 H, s, ring Me), 3.36 (3 H, s, ring Me), 3.34 (3 H, s, ring Me), 3.27 (3 H, s, ring Me), 3.25 (3 H, s, ring Me), 3.20–3.04 (8 H, m, CH_2CH_2CO), 3.20 (3 H, s, ring Me), 3.19 (3 H, s, ring Me), 3.21 (3 H, s, acetyl Me), 3.01 (3 H, s, acetyl Me), 1.40 (9 H, s, Bu^t), 1.38 (9 H, s, Bu^t) and −4.78 (4 H, s, NH).

Separation of 2,4-*Diacetyl*-6-(2-*t*-*butoxycarbonylethyl*)-7-(2-*carboxyethyl*)-1,3,5,8-*tetramethylporphyrin* **10** *and* 2,4-*Diacetyl*-7-(2-*t*-*butoxycarbonylethyl*)-6-(2-*carboxyethyl*)-1,3,5,8-*tetramethylporphyrin* **11**.—The mixture of isomers **10** and **11** (100 mg) was dissolved in THF (10 cm^3), applied to PLC plates (10 mg for each plate) and the plates were then developed in 4.5% MeOH–CH_2Cl_2, dried, and re-run until the two isomers were separated (*ca.* 6 h). The two bands were removed from the plates, suspended in water (50 cm^3), and stirred for 20 min; THF (50 cm^3) was added to the silica/water suspension and this mixture was stirred for 5 min more before the suspension was diluted with chloroform (200 cm^3) and stirred for 2 min. The suspension was transferred to a separatory funnel containing water (500 cm^3); the organic phase was collected and the aq. layer was washed with chloroform (5 × 50 cm^3) until the aq. phase turned a milky white. The combined organic phases were then washed with water (5 × 500 cm^3) to remove silica gel from the organic phase and after removal of the solvent the residue was crystallized from THF–n-hexane to give Band 'A' (fast running fraction) (38 mg) (i.e., **10**) and Band 'B' (slow running fraction) (52 mg) (*i.e.*, **11**) [Found: (mixture of **10** and **11**): C, 68.55; H, 6.9; N, 8.3. $C_{38}H_{42}N_4O_6$·H_2O requires C, 68.25; H, 6.63; N, 8.38%]. 2,4-Diacetyl-6-(2-t-butoxycarbonylethyl)-7-(2-carboxyethyl)-1,3,5,8-tetramethylporphyrin **10**, m.p. >300 °C; δ_H[$(CD_3)_2SO$–$CDCl_3$] 9.85 (1 H, s, meso H), 9.76 (1 H, s, meso H), 9.12 (1 H, s, meso H), 8.84 (1 H, s, meso H), 3.75 (4 H, m, CH_2CH_2CO), 3.17 (3 H, s, ring Me), 3.07 (3 H, s, ring Me), 3.04 (3 H, s, ring Me), 2.93 (3 H, s, ring Me), 2.81 (3 H, s, acetyl Me), 2.67 (3 H, s, acetyl Me), 2.67 (4 H, m, CH_2CH_2CO), 1.01 (9 H, s, Bu^t) and −5.24 (2 H, s, NH); λ_{max}(THF)/nm 420 (ε 135 200), 514 (12 400), 548 (7200), 586 (5900) and 638 (3100). 2,4-Diacetyl-7-(2-t-butyoxycarbonylethyl)-6-(2-carboxyethyl)-1,3,5,8-tetra-

methylporphyrin **11**, m.p. 161.5–163 °C; δ_H[$(CD_3)_2SO$–$CDCl_3$] 9.70 (1 H, s, meso H), 9.50 (1 H, s, meso H), 8.96 (1 H, s, meso H), 8.58 (1 H, s, meso H), 3.73 (2 H, m, $CH_2CH_2CO_2$), 3.68 (2 H, m, CH_2CH_2CO), 3.10 (3 H, s, ring Me), 3.01 (3 H, s, ring Me), 2.88 (3 H, s, ring Me), 2.80 (3 H, s, ring Me), 2.73 (3 H, s, acetyl Me), 2.65 (4 H, m, CH_2CH_2CO), 2.56 (3 H, s, acetyl Me), 0.97 (9 H s, Bu^t) and −5.66 (2 H, s, NH); λ_{max}(THF)/nm 420 (ε 144 100), 514 (14 500), 548 (8400), 586 (7000) and 638 (4200).

Band 'A' **10** and band 'B' **11** were treated individually with excess of ethereal diazomethane. After evaporation of the solvent the products were recrystallized from THF–n-hexane to give 2,4-diacetyl-6-(2-t-butoxycarbonylethyl)-7-(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin **13** and 2,4-diacetyl-7-(2-t-butoxycarbonylethyl)-6-(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin **14**, each in quantitative yield [Found (mixture of **13** and **14**): C, 70.0; H, 6.8; N, 8.3. $C_{39}H_{44}N_4O_6$ requires C, 70.46; H, 6.67; N, 8.43%]; Compound **13** had m.p. 220–221 °C; δ_H($CDCl_3$) 10.39 (1 H, s, meso H), 10.34 (1 H, s, meso H), 9.56 (1 H, s, meso H), 9.33 (1 H, s, meso H), 4.19 (4 H, m, CH_2CH_2CO), 3.65 (3 H, s, ring Me), 3.65 (3 H, s, OMe), 3.51 (6 H, s, ring Me), 3.49 (3 H, s, ring Me), 3.32 (3 H, s, acetyl Me), 3.10 (3 H, s, acetyl Me), 3.07 (4 H, m, CH_2CH_2CO), 1.39 (9 H, s, Bu^t) and −4.47 (2 H, s, NH); λ_{max}(CH_2Cl_2)/nm 420 (ε 130 100), 512 (11 100), 546 (5600), 586 (4200) and 640 (1600). Compound **14** had m.p. 188.5–190 °C; δ_H($CDCl_3$) 10.46 (1 H, s, meso H), 10.43 (1 H, s, meso H), 9.62 (1 H, s, meso H), 9.48 (1 H, s, meso H), 4.22 (4 H, m, CH_2CH_2CO), 3.78 (3 H, s, ring Me), 3.66 (3 H, s, OMe), 3.58 (3 H, s, ring Me), 3.51 (3 H, s, ring Me), 3.40 (3 H, s, ring Me), 3.26 (3 H, s, acetyl Me), 3.16 (3 H, s, acetyl Me), 3.19 (2 H, m, CH_2CH_2CO), 3.06 (2 H, m, CH_2CH_2CO), 1.37 (9 H, s, Bu^t) and −4.27 (2 H, s, NH); λ_{max}(CH_2Cl_2)/nm 422 (ε 135 900), 514 (12 500), 548 (7400), 584 (6300) and 638 (3200).

6-(2-*t-Butoxycarbonylethyl*)-2,4-*bis*-(2-*hydroxyethyl*)-7-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphyrin* **15** *and* (2-*t-Butoxycarbonylethyl*)-2,4-*bis*-(1-*hydroxyethyl*)-6-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphyrin* **16**.—A solution of one of the foregoing 2,4-diacetylmono-t-butyl monomethyl esters **13** or **14** (30 mg) in dry CH_2Cl_2 (10 cm^3) was cooled to 0 °C in an ice-bath and a solution of sodium borohydride (60 mg) in ice-cooled MeOH (5 cm^3) was added. The reaction mixture was stirred at 0 °C under N_2 for 1 h after which time TLC indicated that reduction of the acetyl functions was complete. The solution was stirred briefly with 1 mol dm^{-3} acetic acid (20 cm^3) before being washed with water (2 × 100 cm^3). The organic phase was collected and the solvent was removed under reduced pressure. The residue was crystallized from THF–n-hexane to give the product **15** or **16** (27 mg, 90%) [Found (mixture of **15** and **16**): C, 70.1; H, 7.3; N, 8.3. $C_{39}H_{48}N_4O_6$ requires C, 70.04; H, 7.23; N, 8.34%]. 6-(2-t-Butoxycarbonylethyl)-2,4-bis-(1-hydroxyethyl)-7-(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin **15** had m.p. 193.5–195 °C; δ_H($CDCl_3$) (mixture of diastereoisomers) 10.13 (1 H, s, meso H), 10.11 (1 H, s, meso H), 10.03 (1 H, s, meso H), 10.02 (1 H, s, meso H), 9.80 (1 H, s, meso H), 9.79 (1 H, s, meso H), 9.76 (2 H, s, meso H), 6.14–5.99 [2 H, m, MeC(OH)*H*], 4.16 (8 H, m, CH_2CH_2CO), 3.65 (6 H, s, OMe), 3.44, 3.43, 3.42, 3.41, 3.37, 3.32, 3.31 and 3.28 (each 3 H, s, ring Me), 3.16 (4 H, m, CH_2CH_2CO), 3.08 (4 H, m, CH_2CH_2CO), 2.03–1.94 [12 H, m, *Me*C(OH)H], 1.39 (18 H, s, Bu^t) and −4.24 (4 H, s, NH); λ_{max}(CH_2Cl_2)/nm 400 (ε 169 000), 498 (12 800), 532 (8300), 568 (6200) and 622 (3500). 7-(2-t-Butoxycarbonylethyl)-2,4-bis-(1-hydroxyethyl)-6-(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin **16** had m.p. 184.5–186 °C; δ_H($CDCl_3$) 10.06, 10.05 (1 H, s, s, meso H), 10.01, 10.00 (1 H, s, s, meso H), 9.78, 9.77 (1 H, s, s, meso H), 9.76, 9.75 (1 H, s, s, meso H), 6.02–5.57 [2 H, m, *H*(OH)Me], 4.13 (8 H, m, CH_2CH_2CO), 3.67 (6 H, s, OMe), 3.44, 3.43, 3.42, 3.41, 3.30, 3.29, 3.28 and 3.25 (each 3 H, s, ring Me), 3.20 (4 H, m, CH_2CH_2CO), 3.06 (4 H, m, CH_2CH_2CO), 1.99–1.97 [12 H, m, *Me*C(OH)H], 1.40 (18 H, s, Bu^t) and −4.29, 4.27 (2 H, s, s, NH); λ_{max}(THF)/nm 400 (ε 162 000), 498 (13 200), 530 (8900), 570 (6600) and 622 (4450).

6-(2-*t-Butoxycarbonylethyl*)-7-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethyl*-2,4-*divinylporphyrin* **17** *and* 7-(2-*t-Butoxycarbonylethyl*)-6-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethyl*-2,4-*divinylporphyrin* **18**.—One of the foregoing 2,4-bis-(1-hydroxyethyl) mono-t-butyl monomethyl esters **15** or **16** (25 mg) was dissolved in *o*-dichlorobenzene (5 cm^3) containing PTSA monohydrate (6 mg). The reaction mixture was heated at 100 °C while being monitored spectrophotometrically (reaction solution + triethylamine); the reaction was complete when the peak at 622 moved to 630 nm. The solution was then diluted with CH_2Cl_2 (100 cm^3), washed with water (3 × 150 cm^3) to remove PTSA and the organic phase was collected and the solvent was removed under reduced pressure. The residue was subjected to column chromatography on silica gel, and eluted initially with CH_2Cl_2 to remove small amounts of *o*-dichlorobenzene, and then with 5% MeOH–CH_2Cl_2. The major fraction was collected, then washed with water (5 × 200 cm^3), and the organic phase was collected and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and applied onto thick-layer PLC plates, which were developed with 3% THF–CH_2Cl_2. The major fraction was collected and the solvent was removed under reduced pressure to give a residue, which was crystallized from THF–n-hexane to give compound **17** or **18** (23 mg, 85%) [Found (mixture of **17** and **18**): C, 72.6; H, 7.15; N, 8.6. $C_{39}H_{44}N_4O_4$·CH_3OH requires C, 72.27; H, 7.27; N, 8.43%]. 6-(2-t-Butoxycarbonylethyl)-7-(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2,4-divinylporphyrin **17** had m.p. 175–177 °C; δ_H($CDCl_3$) 9.88 (1 H, s, meso H), 9.80 (1 H, s, meso H), 9.79 (1 H, s, meso H), 9.69 (1 H, s, meso H), 8.18–8.01 (2 H, m, α-vinyl), 6.32–6.09 (4 H, m, β-vinyl), 4.33–4.26 (m, 4 H, $CH_2CH_2CO_2$), 3.69 (3 H, s, OMe), 3.54 (3 H, s, ring Me), 3.51 (3 H, s, ring Me), 3.50 (3 H, s, ring Me), 3.48 (3 H, s, ring Me), 3.23 (2 H, m, $CH_2CH_2CO_2$), 3.14 (2 H, m, $CH_2CH_2CO_2$), 1.43 (9 H, s, Bu^t) and −4.40 (2 H, s, NH); λ_{max}(CH_2Cl_2)/nm 406 (ε 150 200), 504 (13 800), 538 (13 200), 574 (11 400) and 628 (5600). 7-(2-t-Butoxycarbonylethyl)-6-(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2,4-divinylporphyrin **18** had m.p. 139–141 °C; δ_H($CDCl_3$) 10.05 (1 H, s, meso H), 10.03 (1 H, s, meso H), 9.95 (1 H, s, meso H), 9.93 (1 H, s, meso H), 8.28–8.15 (2 H, m, α-vinyl), 6.37–6.14 (4 H, m, β-vinyl), 4.39–4.30 (4 H, m, CH_2CH_2CO), 3.67 (3 H, s, OMe), 3.64 (3 H, s, ring Me), 3.63 (3 H, s, ring Me), 3.59 (3 H, s, ring Me), 3.57 (3 H, s, ring Me), 3.26 (2 H, m, CH_2CH_2CO), 3.16 (2 H, m, CH_2CH_2CO), 1.40 (9 H, s, Bu^t) and −3.99 (2 H, s, NH); λ_{max}(CH_2Cl_2)/nm 406 (ε 163 500), 504 (14 200), 540 (11 650), 574 (6800) and 628 (5200).

7-(2-*Carboxyethyl*)-6-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethyl*-2,4-*divinylporphyrin* **5** *and* 6-(2-*Carboxyethyl*)-7-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethyl*-2,4-*divinylporphyrin* **6**.—One of the foregoing 2,4-divinyl mono-t-butyl monomethyl esters **17** or **18** (20 mg) was stirred with pure TFA (3 cm^3) under N_2 at room temperature for 15 min. The reaction mixture was poured into water (150 cm^3), extracted with CH_2Cl_2 (2 × 100 cm^3) and the organic phase was washed with water (4 × 200 cm^3). After removal of the solvent the title compounds were recrystallized from THF–n-hexane to give compound **5** or **6** (15 mg, 95%) [Found (mixture of **5** and **6**): C, 72.8; H, 6.4; N, 9.7. $C_{35}H_{36}N_4O_4$ requires C, 72.90; H, 6.30; N, 9.72%]; 7-(2-carbonylethyl)-6-(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2,4-divinylporphyrin **5** had m.p. >300 °C; δ_H[$(CD_3)_2$-SO–$CDCl_3$] 9.76 (1 H, s, meso H), 9.75 (1 H, s, meso H), 9.72 (1 H, s, meso H), 9.66 (1 H, s, meso H), 7.98–7.87 (2 H, m, α-vinyl),

6.09–5.89 (4 H, m, β-vinyl), 4.10–4.07 (4 H, m, CH_2CH_2CO), 3.38 (3 H, s, OMe), 3.37 (3 H, s, ring Me), 3.34 (3 H, s, ring Me), 3.32 (3 H, s, ring Me), 3.30 (3 H, s, ring Me), 3.01–2.91 (4 H, m, CH_2CH_2CO) and −4.37 (2 H, s, NH); λ_{max}(THF)/nm 404 (ε 119 700), 504 (11 300), 538 (9000), 576 (5000) and 632 (3800). 6-(2-Carboxyethyl)-7-(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2,4-divinylporphyrin **6** had m.p. >300 °C; δ_H[$(CD_3)_2$SO–$CDCl_3$] 9.52 (2 H, s, 2 × meso H), 9.42 (1 H, s, meso H), 9.40 (1 H, s, meso H), 7.85–7.67 (2 H, m, α-vinyl), 6.01–5.79 (4 H, m, β-vinyl), 4.01–3.95 (4 H, m, CH_2CH_2CO), 3.37 (3 H, s, OMe), 3.21 (6 H, s, 2 × ring Me), 3.19 (3 H, s, ring Me), 3.17 (3 H, s, ring Me), 2.94–2.58 (4 H, m, CH_2CH_2CO) and − 4.85 (2 H, s, NH); λ_{max}(THF)/nm 404 (ε 146 100), 504 (13 600), 538 (10 700), 576 (6100) and 632 (5200).

Dipyridine Magnesium(II)-7-(2-*Carboxyethyl*)-6-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethyl*-2,4-*divinylporphyrin* **2** *and Dipyridine Magnesium*(II)-6-(2-*Carboxyethyl*)-7-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethyl*-2,4-*divinylporphyrin* **4**.—One of the foregoing 2,4-divinylmonomethyl esters **5** or **6** (16 mg) and $Mg(ClO_4)_2$ (400 mg) were refluxed in pyridine (15 cm^3) at 120 °C for 1 h. Spectrophotometry indicated a magnesium(II) complex had formed. The solution was diluted with CH_2Cl_2 (100 cm^3), and washed successively with water (200 cm^3), phosphate buffer (pH 6.8) (2 × 200 cm^3), and again with water (4 × 100 cm^3). After evaporation of the organic solvent the residue was dried under high vacuum. The magnesiated porphyrin **2** or **4** was recrystallized from THF–n-hexane to give the corresponding product (19 mg, 100%). Dipyridine magnesium(II)-7-(2-carboxyethyl)-6-(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2,4-divinylporphyrin **2** had m.p. >300 °C; λ_{max}(THF)/nm 416 (ε 130 200), 550 (10 500) and 590 (8900). Dipyridine magnesium(II) 6-(2-carboxyethyl)-7-(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2,4-divinylporphyrin **4** had m.p. >300 °C; λ_{max}(THF)/nm 424 (ε 100 000), 556 (7800) and 590 (5300).

Biochemical Experiments

Plant Tissue.—Cucumber seeds were germinated in the dark at 30 °C for 6 days. Etiolated seedlings were exposed to white light (60–80 μE m^{-2} s^{-1}) for 20 h.

Plastid Isolation.—Developing chloroplasts were isolated from greening cucumber cotyledons as previously described,[8] except that 'pellet 2' was resuspended in Buffer A (0.5 mol dm^{-3} sorbitol, 1 mmol dm^{-3} $MgCl_2$, 1 mmol dm^{-3} EDTA, 20 mmol dm^{-3} Tes,* 10 mmol dm^{-3} Hepes;* pH 7.7) (5 cm^3 per pellet). The pellet suspension was then illuminated for 5 min at 0 °C to remove remaining traces of endogenous divinylprotochlorophyllide.[3]

Incubation Conditions.—Incubations were carried out in a total volume of 1 cm^3 in Buffer A. Substrates and cofactors were 10 μmol dm^{-3} synthetic porphyrin substrates, 6.5 mmol dm^{-3} glucose-6-phosphate, 0.8 mmol dm^{-3} nicotinamide adenine dinucleotide phosphate (NADPH), 1.1 units of glucose-6-phosphate dehydrogenase; SAM concentration, when added, was 1 mmol dm^{-3}. The samples were incubated in darkness to prevent phototransformation of photodestruction of newly formed divinylprotochlorophyllide. Incubations were carried out at 30 °C for 1 h in a metabolic shaker at 60–65 double strokes min^{-1} and terminated by freezing at −15 °C.

Divinylprotochlorophyllide Extraction and Determination.—Divinylprotochlorophyllide formed during the incubation was extracted into diethyl ether according to the literature method,[9] and was determined using a Perkin-Elmer MP 44-A fluorescence spectrophotometer as previously described.[1]

Protein Determinations.—Protein was determined by the method of Bradford[10] using bovine serum albumin as standard.

* Tes = 2-[tris(hydroxymethyl)methylamino]ethanesulphonic acid and Hepes = 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulphonic acid

Acknowledgements

This research was supported by grants from the National Science Foundation (CHE-90-01381; DCB-88-05668).

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Paper 1/01177D
Received 12*th March* 1991
Accepted 27*th March* 1991