SYNTHESIS AND PHOTOCYTOTOXICITY OF SOME NEW SUBSTITUTED PHTHALOCYANINES

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Abstract—Some new, ring-substituted phthalocyanines have been synthesized and underwent preliminary testing for photodynamic activity using the colony forming ability of Chinese hamster cells in culture as an endpoint. Using 4-(3-N,N-diethylaminopropyl)phthalonitride as a precursor, the previously unknown metal-free 2,9,16,23-tetra-(3-N,N-diethylaminopropyl)phthalocyanine was prepared and converted to its zinc (II) and its cationic water-soluble 2,9,16,23-tetramethylammonium zinc (II) iodide derivatives. Other new phthalocyanine derivatives tested, include a 2,9,16,23-tetra-(2-hydroxymethyl-2-methylbutoxy)phthalocyaninato zinc (II) and 2,3,9,10,16,17,23,24-octahydroxyphthalocyaninato zinc (II) derivatives. Boron tribromide cleavage of the newly prepared and fully characterized 2,3,9,10,16,17,23,24-octamethoxyphthalocyanine gave the unstable metal-free octahydroxyphthalocyanine, which could only be characterized by ultraviolet-visible, NMR and IR spectroscopy.

INTRODUCTION

The phthalocyanine dyes have been recently suggested as sensitizers in the photodynamic therapy of cancer (Ben-Hur and Rosenthal, 1985). The relationship between chemical structure and ability to act as photodynamic sensitizers has not yet been entirely defined. Two photobiologically critical parameters are affected by the structural diversity: (a) the lifetime of the excited triplet state and (b) the cellular uptake of the dye. Both these parameters depend on the nature of the central metal atom. Since a long life triplet state is a prerequisite for photosensitization, a phthalocyanine dye containing a diamagnetic metal is better suited for this function than an analog compound containing a paramagnetic metal (Rosenthal et al., 1986). Furthermore, preliminary screening indicates that metals higher than trivalent, like Si, which require out-of-plane counterions on both sides of the phthalocyanine ring, are not taken up by cells, most probably due to the physical hindrance induced by the axial substituents (Ben-Hur et al., 1987). A similar explanation is provided for the absence of photodynamic activity of phthalocyanine substituents with bulky peripheral substituents such as neopentoxy groups. In addition, the ring substituents, which define the solubility features of the dye, will affect the rate of uptake, as exemplified by the comparison between unsubstituted, tetrahydroxy, and tetransulphonated derivatives (Rosenthal et al., 1987; Brasseur et al., 1988).

We have now prepared metal-free 2,9,16,23-tetra-(3-N,N-diethylaminopropyl) phthalocyanine (1), its zinc (II) (2), and its quaternized tetramethyl zinc (II) iodide (3) derivatives. Other unusual phthalocyanines tested in this study are 2,9,16,23-tetra-(2-hydroxymethyl-2-methylbutoxy) phthalocyaninato zinc (II) (4) (Leznoff et al., unpublished data) and 2,3,9,10,16,17,23,24-octahydroxyphthalocyaninato zinc (II) (5) prepared from 2,3,9,10,16,17,23,24-octamethoxyphthalocyaninato zinc (II) (6). It was expected that the cationic water-soluble phthalocyanine 3, the partially hydrophilic-lipophilic phthalocyanines 2 and 4, and the catechol-like phthalocyanine 5 would provide new and interesting candidates for photodynamic sensitization.

MATERIALS AND METHODS

Procedures for synthesis

Instrumental methods and equipment used in the characterization of new phthalocyanines have been previously outlined (Marcuccio et al., 1985).

Preparation of 4-(3-N,N-diethylaminopropyl)phthalonitrile (9). To a solution of 1.0 g (3.94 mmol) of 4-iodophthalonitrile (7) (Marcuccio et al., 1985; Keller and Griffith, 1980) in 40 ml of dry, freshly distilled diethylamine were added 0.6 g (5.40 mmol) of 3-N,N-diethylanino prop-1-ynyl (8) (Wolf, 1952), 5.0 mg (0.026 mmol) of copper (I) iodide, and 40 mg (0.06 mmol) of bis(triphenylphosphate)palladium dichloride under an Ar atmosphere, using a recent procedure (Takahashi et al., 1980). The reaction mixture was stirred at room temperature for 24 h, during which a precipitate of diethylamine hydroiodide appeared. Thin layer chromatography showed traces of 7, so small amounts of copper(I) iodide, bis(triphenylphosphate)palladium dichloride and 3-N,N-diethylaninoprop-1-ynyl were added, and the mixture was stirred for another 3 h. The mixture was filtered to separate the precipitated salt. The salt was washed with diethyl ether and the filtrate concentrated on the rotary evaporator. The residue was extracted with diethyl ether then

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washed with water (2 × 100 mL), 5% NaHCO₃ (2 × 100 mL) and water (2 × 100 mL). The ether fraction was dried over anhydrous magnesium sulfate and then concentrated on the rotary evaporator. The residue was purified by column chromatography using basic alumina (Act 2-3) and hexane/ether (3:7) as the eluting solvents. Evaporation of the solvent left a yellow oil which was stored under Ar in the refrigerator overnight to give 0.83 g (89% yield) of a yellow solid (9). Recrystallization from ethyl acetate/hexane produced long, yellow, needle-like crystals, mp 45-45°C, IR spectrum (KBr pellet): 3120–2820, 2240, 1600, 1540, 1380, 1350, 1280, 1260, 1190, 1160, 1120, 910, 850, 770 cm⁻¹. 1H NMR spectrum (300 MHz, CDCl₃): δ 8.71–7.73 3H, 3.68 2H, 2.65–2.57 4H, 1.14–1.09 6H. 13C NMR spectrum (300 MHz, CDCl₃): δ 135.60, 135.78, 133.36, 129.29, 116.11, 115.08, 114.69, 114.10, 93.23, 81.70, 47.39, 41.53, 12.52. Mass spectrum: m/z 237 (M⁺), 222, 128. Analysis: C₇H₅N₂. Calc. (%): C(75.90), H(5.37), N(17.70). Found (%): C(75.95), H(5.97), N(17.42).

Preparation of 4-(3-N,N-dimethylaminopropyl)phthalonitrile (10). A solution of 2.4 mg (0.13 mmol) of 9 in 100 mL of dry ethyl acetate was hydrogenated over 10% palladium on charcoal, at 60 psi, in a Parr hydrogenation bottle for 1 h at room temperature. The solution was filtered from the catalyst and concentrated. Column chromatography of the product on 80 g of basic alumina (Act 3) using hexan/ether (3:7) as the eluent, gave up on evaporation of the solvent, 1.9 g (78% yield) of a yellow oil, bp 173–175°C/0.05 torr. IR spectrum (neat, NaCl disc): 2980–2700, 2250, 1600, 1440, 1340, 1290, 1200, 1070, 910, 840, 760 cm⁻¹. 1H NMR spectrum (300 MHz, CDCl₃): δ 8.73 1H, 6.76 1H, 7.59 1H, 2.77 2H, 2.53 4H, 1.84 2H, 1.04 6H. 13C NMR spectrum (300 MHz, CDCl₃): δ 149.24, 133.48, 133.32, 133.26, 115.59, 115.48, 115.43, 112.86, 51.59, 46.56, 39.32, 29.85, 11.42. Mass spectrum: m/z 241 (M⁺), 226, 141. Analysis: C₉H₁₀N₂. Calc. (%): C(74.75), H(8.97), N(17.34). Found (%): C(75.04), H(8.39), N(17.75).

Preparation of 5-(3-N,N-dimethylaminopropyl)-1,3-diimino inoisoindoline (11). For a solution of 200 mg of sodium in 100 mL of dry methanol, was added 1.8 g (7.47 mmol) of 10. The mixture was stirred for 1 h at room temperature, and for 4 h under reflux conditions, during which ammonia gas was bubbled into the solution. The reaction mixture gradually turned a dark green color. The reaction was cooled to room temperature and the addition of ammonia was discontinued. Evaporation of the solvent gave crude 11, which was used directly in a condensation reaction without further purification. Compound 11 did not exhibit a nitrite absorption in its IR spectrum.

Preparation of 2,9,16,23-tetra(3-N,N-dimethylaminopropyl) phthalocyanine (1). The crude 5-(3-N,N-dimethylaminopropyl)-1,3-diiminoisoindoline (11), made from 1.8 g (7.47 mmol) of 10, underwent self-condensation as previously described (Lazznoff et al., 1985). IR spectrum (KBr pellet): 3450, 2940, 1610, 1490, 1340, 1090, 830, 750 cm⁻¹. Mass spectrum: m/z 1010 (M⁺). Absorption spectrum: λmax (chloroform) 675, 672, 616, 352, 246 (log ε: 5.04, 4.93, 4.41, 4.74, 4.45).

Preparation of 2,9,16,23-tetra(3-N,N-dimethyl-N-methyl phthalocyaninato) zinc (II) tetraiodide (3). To a solution of 84 mg (0.81 mmol) of 2 in 10 mL of toluene was added 0.5 mL (8 mmol) of methyl iodide. This solution was stirred for 20 h at room temperature. The observed precipitate was filtered and washed with ether. The yield of the green powder solid 3 was 96.5 mg (74.2%). Absorption spectrum: λmax (water) 634, 338, 290, 226, (log ε: 4.89, 4.84, 4.50, 5.00). IR spectrum (KBr pellet): 3440, 2960, 1610, 1490, 1340, 1090, 1050, 750 cm⁻¹. Analysis: C₉H₁₀N₂-ZnI₄, Calc. (%): C(68.09), H(5.55), N(10.51), Zn(4.09). Found (%): C(48.10), H(5.48), N(10.54), Zn(3.97).

Preparation of 2,3,9,10,16,17,23,24-octahydroxophthalocyaninato zinc (II) (6). To 1.0 g (5.39 mmol) of 1,2-dicyano-4,5-dimethoxybenzene (Metz et al., 1984) in 10 mL of dry cold label dimethylylformamide (DMF), was added 1.01 g (5.53 mmol) of anhydrous zinc nitrate (Pawlowski and Hanack, 1980). After 120 h heating at 160°C (oil bath) under Ar, the mixture was cooled to room temperature and the DMF was removed under reduced pressure (40°C, 0.1 torr). The crude product was filtered and washed with methanol to remove brown impurities. The residue was then extracted with methanol in a Soxhlet apparatus until the extract was clear (1.8 mL) by extraction with acetone. It was then dried at 80°C overnight to give 420 mg (38%) of 6 (Meier et al., 1986) as a dark blue solid. Absorption spectrum: λmax (pyridine) 680, 672, 610, 364 (log ε: 5.20, 5.25 at room temperature). IR spectrum (KBr pellet): 2930, 1600, 1430, 1395, 1275, 1095, 875, 740 cm⁻¹. Mass spectrum: m/z 816, 817, 818, 819, 820, 821.

Preparation of 2,3,9,10,16,17,23,24-octahydroxophthalocyaninato zinc (II) (5). To 206.5 mg (0.25 mmol) of 6 in 50 mL of dry benzene, was added 5 mL (52.6 mmol) of boron tribromide at 10°C, under an Ar atmosphere. The mixture was warmed to room temperature and then refluxed under argon for 20 h. After cooling to room temperature, ice cold water was added and the water–benzene mixture was evaporated under reduced pressure. The crude product was filtered, washed with water and air dried to give 1.85 g of a black-grey powder. Further purification of the product was achieved by extraction with o-acetophenone in a Soxhlet apparatus for 24 h. This process removed 880 mg of water material. The acetoni- rile extraction was repeated until all of the impurities were
removed (an additional 560 mg of off-white powder). The amount of 5 recovered after Soxhlet extractions was 146 mg (82%). A sample of 25.8 mg was further purified by recrystallization from DMSO/CH$_2$Cl$_2$ to yield 16.0 mg of 5 (62% recovery). Absorption spectrum: $\lambda_{max}$ (10% DMF in ethanol) 682, 674, 622, 354, 396 (log $e$: 4.78, 4.70, 4.12, 4.60, 4.49). $^1$H NMR spectrum (300 MHz, DMSO-d$_6$); 8 10.1 (broa signal) $^{13}$C, 80.6. IR spectrum (KBr pellet): broad band from 3100 to 3500 cm$^{-1}$ OH, 1615, 1470, 1380, 1060, 1030, 880 cm$^{-1}$. Mass spectrum: m/z 704, 705, 706, 707, 708, 709.

Preparation of 2,9,10,16,17,23,24-octamethoxyphthalocyanine (12). Ammonia was bubbled into a solution of 1.88 g (10.0 mmol) of 1,2-dicyano-4,5-dimethoxybenzene (Metz et al., 1984) in dry methanol containing sodium methoxide, for 1 h at 85°C and for 8 h under reflux conditions, similar to, but not identical with conditions previously described (Leznoff et al., 1985). Evaporation of the solvent gave the crude 5,6-dimethoxy-1,3-diminoisoindoline (which did not exhibit any CN absorption peaks in its infrared spectrum), which was dissolved in 20 ml of 2-N,N-dimethylaminoethanol and heated to 165°C (oil bath) under an argon atmosphere. After 48 h reflux, the mixture was cooled to room temperature and the solvent was evaporated under reduced pressure. The crude product was digested with methanol containing large amounts of ether, filtered and washed with ether followed by increasing amounts of methanol in ether (1:9) to (1:1), and finally it was washed with methanol and air dried overnight to give 924 mg of a very fine purple powder. The product was then extracted with methanol in a Soxhlet apparatus for 20 h followed by acetone to give 790 mg (42%) of 12 as a dark blue solid. Absorption spectrum: $\lambda_{max}$ (CF$_2$CO$_2$H) 734, 702, 406, 292 (log $e$: 5.14, 4.84, 4.64, 4.95). IR spectrum (KBr pellet): 3285, 2920, 1600, 1475, 1270, 1195, 1095, 1015 cm$^{-1}$. Mass spectrum: m/z 754, 739, 724, 709, 694, 679. Analysis: C$_{29}$H$_{29}$N$_7$O$_7$, Calc.: (%) C(63.66), H(4.54). Found (%): C(63.28), H(4.84). (14.77).

Preparation of 2,9,10,16,17,23,24-octachloro-phthalocyanine (13). To 40 mg (0.033 mmol) of 12 in 20 ml of dichloromethane, was added 0.3 mg (3.17 mmol) of boron tribromide at 0°C under an argon atmosphere. The mixture was warmed to room temperature and then refluxed under Ar for 22 h. After cooling to room temperature, 10 ml of distilled water was added and the suspension was stirred for 10 min. The crude product was filtered under suction and washed with water, ether and air dried. Exhaustive Soxhlet extractions with methanol and then ether gave 23.0 mg (67%) of 13 as a dark blue solid. Absorption spectrum: $\lambda_{max}$ (pyridine) 708, 678, 672, 652, 612, 428, 350 (log $e$: 4.72, 4.61, 4.65, 4.27, 4.08, 4.42, 4.57). $^1$H NMR spectrum (300 MHz, DMSO-d$_6$); 8 8.6, 10.1 (broad signal) $^{13}$C, 80.6. IR spectrum (KBr pellet): 3280 (broad), 1605, 1475, 1380, 1260, 1058, 1015, 850, 740 cm$^{-1}$.

Preparation of 2,9,16,23-tetra-(3-N,N-dialkyl-N-methyaminopropyl)phthalocyanine tetraiodide (14). Methyl iodide (0.5 ml, 8 mmol) was added to a solution of 34 mg (0.033 mmol) of 1 in 25 ml of ethyl ether. This mixture was stirred for 48 h at room temperature. The above observed precipitate was filtered and dried to give 48 mg (89%) yield of 14 as a dark blue powder. Absorption spectrum: $\lambda_{max}$ (H$_2$O) 639, 332, 292, 226 (log $e$: 4.75, 4.60, 4.00, 4.92). IR spectrum (KBr pellet): 3440, NH, 2980, 1610, 1390, 1320, 1100, 1010, 810, 750 cm$^{-1}$. Analysis: C$_{39}$H$_{39}$IN$_{14}$, Calc (%): C(50.07). Found (%): C(50.19), H(5.82), N(11.67).

Preparation of 2,9,16,23-tetra-(N,N-dialkylaminopropyl)phthalocyanine tetrahydrochloride (15). Hydrogen chloride gas was bubbled into a solution of 45.5 mg (0.47 mmol) of metal free phthalocyanine 12 in 25 ml of ethyl ether. This mixture was stirred for 15 min at room temperature. The observed dark blue solid precipitate was filtered and dried to yield 47.1 mg (90% yield) of 15.

RESULTS AND DISCUSSION

Syntheses

The preparation of the aminophthalocyanines 1 and 2 and the tetramethyl derivative 3 have not been previously described. Thus, as shown in Fig. 1, treatment of 4-iodophthalonitrile (II) with 3-N,N-diethylaminomethyl-4-dicyano-1-ynylphthalonitrile (9) in 95% yield. Catalytic hydrogenation of 9 gave 4-(3,N,N-diethylaminomethyl-1-ynyl)phthalonitrile (10) in 78% yield. Conversion of 10 into 5-(3,N,N-diethylaminomethyl)-1,3-diminoisoindoline (11) was achieved by bubbling ammonia into a solution of 10 and sodium methoxide, in methanol. Compound 11 underwent self-condensation when heated in 2-N,N-dimethylaniline to give the dark blue 2,9,16,23-tetra-(3-N,N-diethylaminomethyl) phthalocyanine (1) in 28% yield after purification by chromatography. Insertion of zinc into metal-free 1 was readily attained by refluxing 1 with anhydrous zinc acetate in 1:1 toluene/2-methoxyethanol to give 2,9,16,23-tetra-(3-N,N-diethylaminomethyl)phthalocyaninato zinc (II) (2). Quaternization of the four amino groups of 2 occurred on treatment of 2 with excess iodomethane in toluene at room temperature to give 2,9,16,23-tetra-(3-N,N-diethyl-methylaminomethyl)phthalocyaninato zinc (II) tetraiodide (3) in 97% yield. The synthesis of 2,9,16,23-tetra-(2-hydroxymethyl-2-methylbutoxy)phthalocyaninato zinc (II) (4) and related compounds will be reported elsewhere (Leznoff et al., unpublished data). It should be noted that 1-4, each exist as a closely related mixture of isomers containing the depicted structures (Fig. 1) and their 2,9,16,23-, 2,9,17,24- and 2,10,16,24- derivatives.

Chinese hamster cells and light exposure

Chinese hamster fibroblasts, line V79-B31OH, were grown as a monolayer in 10 cm plastic Petri dishes. Growth was in Dulbecco's modified Eagle medium containing 10% fetal calf serum, at 37°C in a humidified atmosphere adjusted to 5% CO$_2$. The detailed experimental procedure for determination of phototoxicity was described by Rosenthal et al. (1987).
earlier preparation of 2,9,16,23-tetrahydroxyphthalocyaninato zinc (II) (Rosenthal et al., 1987) gave the previously unknown 2,3,9,10,16,17,23,24-octamethoxyphthalocyaninato zinc (II) (5) as a fairly insoluble dark blue compound. Although metal-free 2,3,9,10,16,17,23,24-octamethoxyphthalocyanine (12) has now been prepared for the first time (see Materials and Methods), boron tribromide cleavage of 12 produced a highly insoluble material (slightly soluble in dimethyl sulfoxide or pyridine) which exhibited ultraviolet-visible, infrared, and NMR spectra characteristic of the metal-free, 2,3,9,10,16,17,23,24-octahydroxyphthalocyanine (13), but mass spectral and elemental analysis were unsatisfactory. Thus attempts at making 5 by zinc insertion into 13 could not be achieved. In addition, zinc insertion into 12 in order to make 5 was incomplete due to the insolubility of 12.

Photocytotoxicity

The photodynamic activity of these previously unknown phthalocyanines have been estimated using the colony forming ability of Chinese hamster cells as an endpoint. Since other metal-free phthalocyanines showed minimal photodynamic activity (Chan et al., 1987; Abernathey et al., 1987; Sonoda et al., 1987), at the present only zinc-containing derivatives were assayed. The results are displayed in Fig. 2.
It was suggested that the photodynamic activity of phthalocyanines is expressed in membrane deterioration (Ben-Hur and Rosenthal, 1986). Since charged groups impart a net surface charge to most biological membranes, this surface charge might be expected to affect the association of the sensitizer with the membranes, either at a specific site, or globally. Thus, mitochondria accumulate positively charged lipophilic dyes, apparently due to the transmembrane potential which is 100–300 mV negative inside the organelle; up to 10^4 fold concentration gradient is theoretically possible. Mitochondria of carcinoma cells take up higher concentration of cationic molecules, and retain them longer than most normal cells. Thus photodynamically active cationic dyes may serve as carcinoma cell-specific phototoxic agents (Oseroff et al., 1986).

Up to now, only neutral or negatively charged phthalocyanines have been tested. Compound 3 is a cationic, water-soluble, phthalocyanine, containing a tetraalkylammonium group at the remote end of the lipophilic side chains. However, this cationic phthalocyanine was less toxic to mammalian cells in culture than its neutral counterpart. The present results point to 2 as the most active compound in this group of new dyes. Compounds 4 and 5 displayed very little phototoxicity. The octahydroxy derivative 5 is therefore inferior to its tetrahydroxy analog which possesses high photobiological activity (Rosenthal et al., 1987). This is presumably due to the decreased uptake of 5 into the cells, either because of the greater substitution and consequent steric hindrance, or lower solubility which is characteristic of highly symmetrical derivatives.

While these results are indicative of general phototoxicity, it remains to be seen if the efficiency of the cationic compound 3 is enhanced toward carcinoma cells in vivo.

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REFERENCES


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