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Note

Synthesis of 5-[*o*-(10-phenylisoalloxazine-3-acetamido)phenyl]-10,15,20-triphenylporphyrin

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Synthesis and structure of 5-[*o*-(10-phenylisoalloxazine-3-acetamido)phenyl]-10, 15, 20-triphenylporphyrin is described. The fluorescence spectra of above compound in different organic solvents, surfactant solution are reported.

Flavin coenzyme and heme coenzyme are present together in many enzymes including NADPH-sulphite reductase[1,2], NADPH-nitrite reductase[3] and NADPH-nitrate reductase[4] as well as proteins such as cytochrome c_{552}[5], cytochrome c_{553}[6], lactate dehydrogenase[7] and yeast haemoglobin[8]. Electron transfer between flavin coenzyme to heme coenzyme occurs via inner and outer sphere mechanism[9,10]. Recently, we have reported the synthesis of tetramethylene diamine 8,8′-bis(riboflavin tetracetate) and their incorporation in DMPC vesicles[11]. Herein we report the synthesis and structure of flavin linked porphyrin with an aim to understand the molecular mechanism of electron transfer from flavin to the heme system.

Results and Discussion

The cyclocondensation of 2-nitro-N-phenylaniline with alloxan monohydrate in acidic conditions gives the 10-phenylisoalloxazine[12-14] (**1**) which on reaction with ethyl bromoacetate gives ethyl 10-phenylisoalloxazine-3-acetate (**2**) in 73% yield. The hydrolysis of **2** with conc. HCl gives 10-phenylisoalloxazine-3-acetic acid (**3**). The cyclocondensation of *o*-nitrobenzaldehyde and benzaldehyde with pyrrole in propionic acid followed by repeated column chromatography gives the 5-(*o*-nitrophenyl)-10, 15, 20-triphenylporphyrin which on reduction with $SnCl_2$/HCl gives 5-(*o*-aminophenyl)-10, 15, 20-triphenylporphyrin[15] (**4**). The reaction of **4** with 10-phenylisoalloxazine-3-acetic acid (**3**) by mixed anhydride method gives 5-[*o*-(10-phenylisoalloxazine-3-acetamido)phenyl]-10, 15, 20-triphenylporphyrin (**5**) (Scheme I) in 41% yield. The UV-visible spectra of flavin covalently linked with porphyrins are comparable to one reported for flavin linked porphyrins[16,17]. Further the structures of compounds were confirmed by UV-visible, IR, ^{1}H NMR and FAB MS spectral data.

The fluorescence spectra of isoalloxazine[18-20] and porphyrins[21] are used as probe for medium polarity and microenvironment. Fluorescence spectra of **1, 4** and **5** (5×10^{-5} *M* in $CHCl_3$) show decrease in fluorescence intensity of **5** in comparison to **1** and **4** (Figure 1). Sharp decrease in relative intensity or quenching of λ_{max} at 654 and 717 nm in the fluorescence spectra of **5** (5×10^{-7} *M*) is observed. Fluorescence spectra of **5** (5×10^{-5} *M*) in methanol solution gave λ_{max} 506(95), 529.2(85), 605(100), 654.6(240) and 720.6(60) nm. The fluorescence intensity of **5** increases in 10 m*M* sodium dodecyl sulphate solution as compared to methanol solution. The change in intensity of flavins in surfactant solution

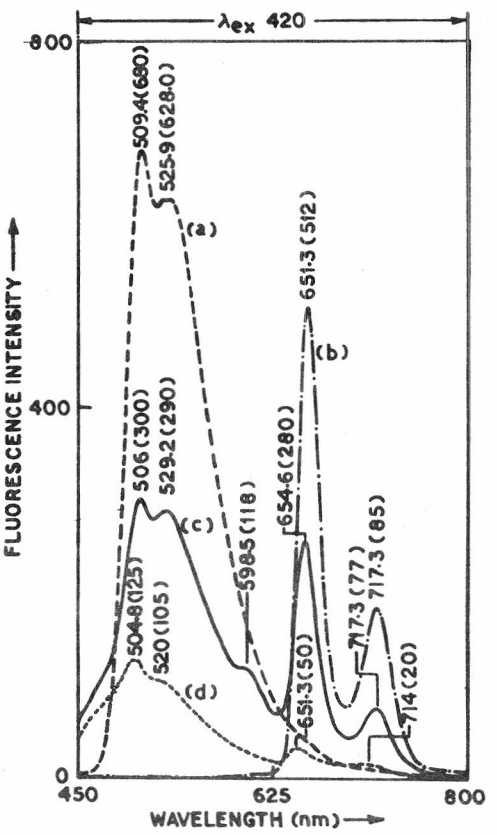
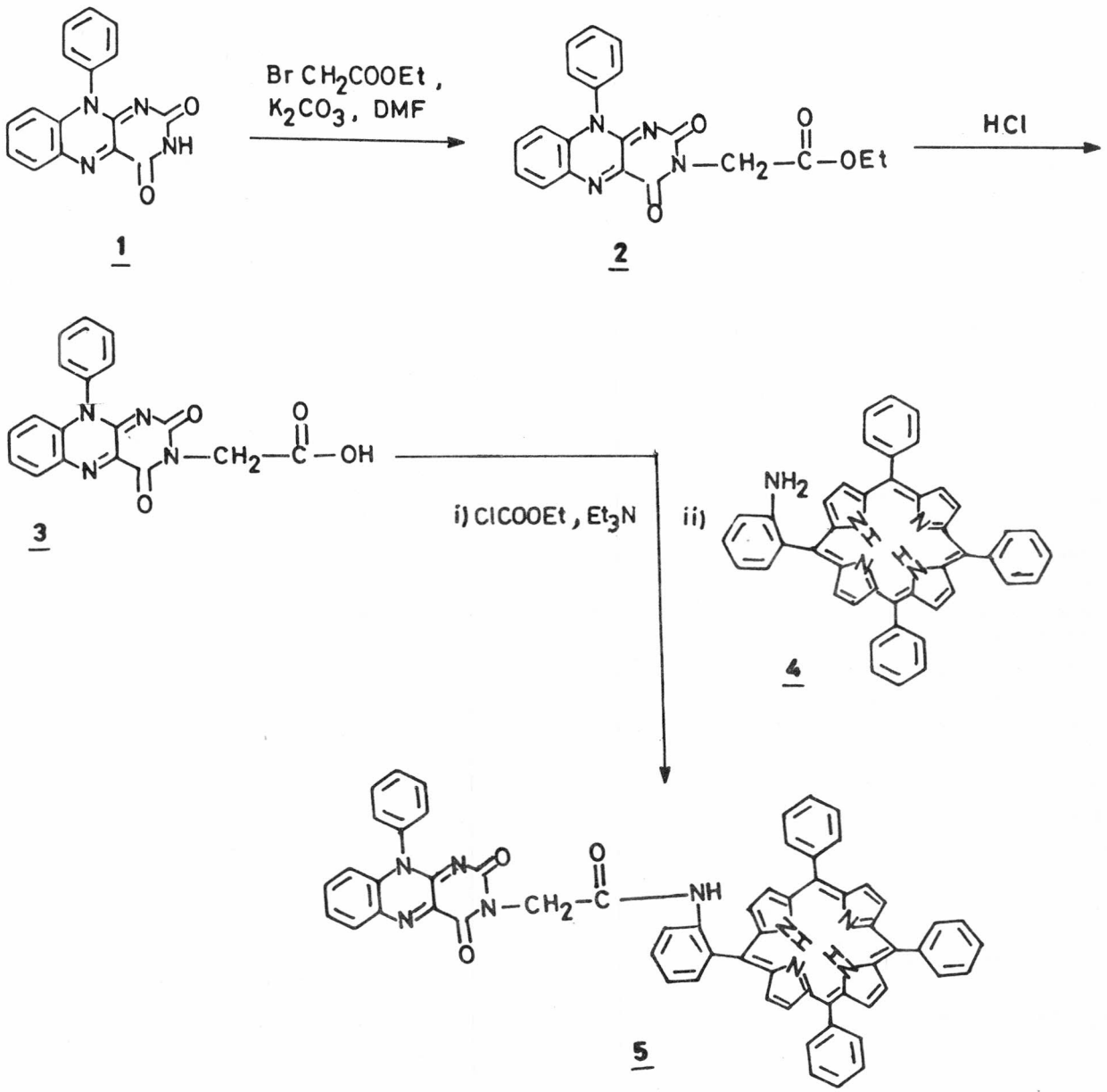


Figure 1—Fluorescence spectra of **1**(a), **4**(b), **5**(c) (10^{-5} *M*) and **5**(d) (10^{-7} *M*) in chloroform



Scheme I

has been reported due to monomerization effect[22].

The detailed studies regarding the eletron transfer and aggregational properties of different covalently linked porphyrin flavin are in progress and results will be reported elsewhere.

Experimental Section

All melting points were determined on Thomas Hoover Unimelt Capillary apparatus. IR spectra were recorded on Perkin Elmer FT-1710 or Shimadzu IR-435 spectrophotometer (ν_{max} in cm^{-1}). Absorption spectra were recorded on Shimadzu UV-260 spectrophotometer and absorption maxima are expressed in nm. Fluorescence spectra were recorded on Jobin-Youn Jy-3.c.s. spectrofluorimeter and emission maxima are expressed in nm. ^1H NMR were recorded on Jeol-FNM FX 200 (200 MHz) or Perkin Elmer R-32 (90 MHz) using TMS as an internal reference (chemical shift in δ, ppm). Mass spectra was recorded on Jeol SX-102 (FAB) spectrometer using xenon as the FAB gas and *m*-nitrobenzyl alcohol was used as the matrix.

Starting materials. 2-Nitro-N-phenylaniline (m.p. 72-74°C, lit[13,14] m.p. 72-74°C), 10-phenylisoalloxazine (**1**) (m.p. > 290°C, lit[23] m.p. > 300°C) and 5-(*o*-amino phenyl)-10, 15, 20-triphenyl-porphyrin[15] (**4**) were prepared and characterised by the modification of reported procedures.

Ethyl 10-phenylisoalloxazine-3-acetate (2). Ethyl bromoacetate (2.8 g, 16.8 mmole) was added to the suspension of **1** (1.27 g, 4.38 mmole) and anhyd. K_2CO_3 (3.4 g, 24.6 mmole) in DMF. The reaction mixture was stirred at room temperature for 5 h. Potassium carbonate was filtered off and solvent was removed under reduced pressure. Water (150 mL) was then added and residue was extracted with chloroform. The combined organic phase was dried (Na_2SO_4) and solvent was evaporated to give ethyl 10-phenylisoalloxazine-3-acetate in 73% (1.2 g) yield; m.p. > 270°C; IR(KBr): 1733, 1712, 1662, 1587, 1613, 1579, 1481, 1479, 1030, 808, 770, 753 and 700; ^{1}H NMR (DMSO-d_6): 1.22 (t, 3H, J = 6 Hz, $-OCH_2CH_3$), 4.02-4.27 (q, 2H, J = 7 Hz, $-OCH_2CH_3$), 4.67 (s, 2H, N-CH_2), 6.6-6.82 (dd, 1H, J_{9H-8H} = 8 Hz, J_{9H-7H} = 2 Hz, H-9), 7.07-7.17 (m, 2H, H-7,8), 7.42-7.52 (m, 5H, N-aryl-H), 8.02-8.12 (dd, 1H, J_{6H-7H} = 8 Hz, J_{6H-8H} = 2 Hz, H-6).

10-Phenylisoalloxazine-3-acetic acid (3). Compound **2** (0.9 g, 2.58 mmole) was dissolved in conc. HCl (10 mL) and the reaction mixture was stirred at 80-90°C for 45 min. It was then cooled and ice cold water was added to it. Yellow crystals were filtered by suction to give 10-phenylisoalloxazine-3-acetic acid in 62% (520 mg) yield; m.p. 205°C; IR(KBr): 3414-2300, 1729, 1670, 1558, 1453, 1227 and 696; ^{1}H NMR (DMSO-d_6): 4.88 (s, 2H, N-CH_2), 6.8-6.86 (dd, 1H, J_{9H-8H} = 8 Hz, J_{9H-7H} = 2 Hz, H-9), 7.58 (m, 2H, H-7 and H-8), 7.74-7.84 (m, 5H, N-aryl-H), 8.26-8.34 (dd, 1H, J_{6H-7H} = 8 Hz, J_{6H-8H} = 2 Hz, H-6).

5-[*o*(10-Phenylisoalloxazine-3- acetamido)phenyl]-10, 15, 20-triphenylporphyrin (5). Ethyl chloroformate (5.6 g, 52 mmole) was added on portions to the solution of dry triethylamine (1 mL) and **3** (0.34 g, 1 mmole) in chloroform (250 mL), and finally chloroform solution of **4** (0.63 g, 1 mmole) was added dropwise maintaining the temperature at 0°C. The reaction mixture was stirred for 24 h at room temperature and was washed with sodium bicarbonate solution followed by water. The organic layer was dried (anhyd. Na_2SO_4) and solvent was evaporated under reduced pressure. The residue was chromatographed on silica gel and eluted with chloroform and finally elution with chloroform: acetone (7:3) gave **5** in 41% (0.39 g) yield; UV-visible ($CHCl_3$) (ε_{max} in mm): 272 (0.62), 419 (1.75), 514 (0.5), 546 (0.4), 588 (0.32) and 648.6 (0.2); IR(KBr): 3000, 1720, 1680, 1560, 1210, 1085, 1015, 862, 800 and 700; ^{1}H NMR ($CDCl_3$): 3.50 (s, 2H, internal py NH), 4.25 (s, 2H, CH_2), 5.50, 5.80, 7.26, 7.60-7.80 (m, 29H, NH and aryl proton), 8.30-8.50 (m, 8H, pyrrolic-βH); FAB MS (m/z): 960 (M+1), 599 (100%, Base peak).

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