Model Studies on the Active Site of Cytochrome P-450: an Fe^{II}–Porphyrin carrying a Strapped Thiolate Ligand

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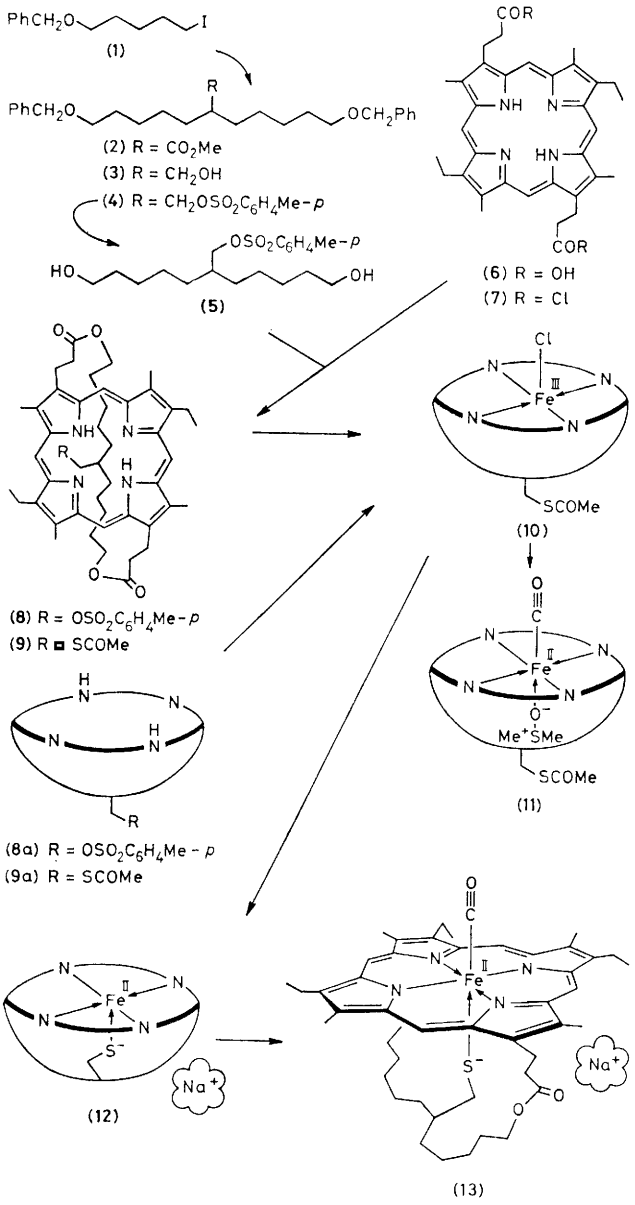
An Fe^{II}–porphyrin has been synthesised with a strap carrying a thiolate residue covalently bound across one face of the macrocycle; the spectroscopic properties of the carbon monoxide complex of this model system closely match those of the CO-complex of cytochrome P-450.

The ability of cytochrome P-450 to activate molecular oxygen for mild, specific oxidation of organic substrates[1] is one which chemists must admire. There is obvious fundamental and practical interest in designing simpler model systems with the long-term aim of carrying out similar oxidations, even if less efficiently, and there has been considerable recent interest in this area.[2]

The active site of cytochrome P-450 contains an iron



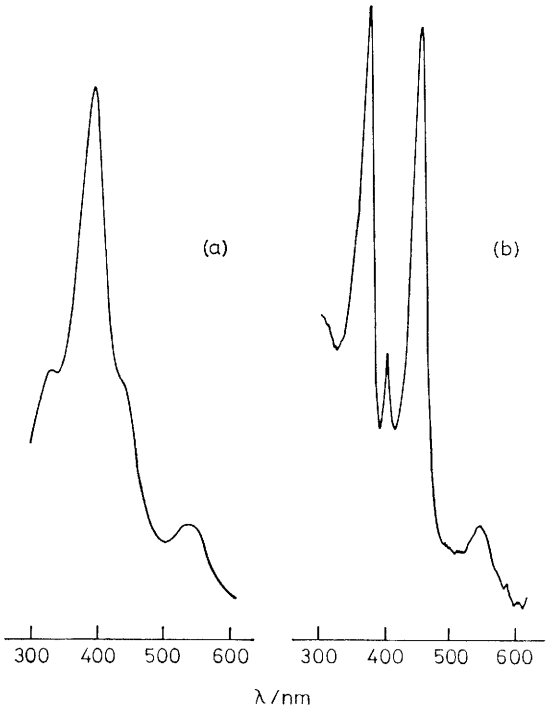


Figure 1. U.v.–visible absorption spectra in DMSO (a) of the penta-co-ordinate Fe^{II}–complex (**12**) and (b) of the hexa-co-ordinate Fe^{II}–CO complex (**13**).

porphyrin and there is strong evidence[2e] that a thiolate group co-ordinated to the iron is important for at least some stages of the catalytic cycle. One of the problems in designing a model system is how to promote co-ordination of the thiolate residue to the metallic centre. In earlier studies, either a large excess of the added thiolate has been used (10^2—10^4 equiv.)[2e] or a 'tail' attached covalently at one end to the porphyrin nucleus has carried the thiolate group.[2a] In the work outlined here, we aimed to hold the thiolate firmly in a suitable position for binding by strapping it across one face of the porphyrin macrocycle through two covalent attachments to the periphery. This approach had been successful in holding the basic ligand for co-ordination to iron in the doubly-bridged model which reversibly binds oxygen at ambient temperatures.[3]

It was also necessary that the synthetic plan should avoid having Fe^{III} and R–SH present in the same system since the former oxidises the latter to disulphide. The sulphur was therefore to be protected until the iron had been reduced to Fe^{II}, an approach also used independently.[2a]

The ester† (**2**), prepared by standard steps from the iodide† (**1**) and dimethyl malonate, was reduced with lithium aluminium hydride to the alcohol† [(**3**), 82%]. The benzyl groups were cleaved from the derived toluene-*p*-sulphonate† [(**4**), 93%] by hydrogenation over palladised charcoal to yield the diol† [(**5**), 91%]. Mesoporphyrin-II (**6**), prepared as earlier,[4] was converted into its soluble acid chloride (**7**) (using oxalyl chloride) and this reacted under high dilution conditions with the diol (**5**) at room temperature to yield the strapped system† [(**8**), 25%], m.p. 188—191 °C. This substance is also illustrated in diagrammatic form as (**8a**) and this device is used for most of the sequel. Displacement of the tosyloxy group from (**8**)/(**8a**) using potassium thioacetate afforded the required sulphur-carrying porphyrin† in protected form [(**9**)/(**9a**), 78%], m.p. 172—175 °C.

Iron insertion ($FeSO_4$ method) occurred readily into the porphyrin (**9a**) and the product was isolated as its chloride derivative† [(**10**), 77%], m.p. 236—238 °C. This was reduced in dimethyl sulphoxide with the complex of 18-crown-6-ether and sodium dithionite[5] all in a glove-box (<4 p.p.m. O_2) and part of the product was characterised (n.m.r. and u.v.–visible spectroscopy) as its complex with carbon monoxide (**11**). The remainder was treated with dimsyl sodium to cleave the *S*-acetyl group and generate the penta-co-ordinated Fe^{II} system (**12**) having a u.v.–visible spectrum (Figure 1) closely similar in form to that of the reduced form of P-450 from *Pseudomonas putida* ($P\text{-}450_{cam}$);[6] as expected, the absorption of (**12**) was at slightly shorter wavelength (*ca.* 10 nm) owing to lack of the vinyl groups present in the natural haem. The product (**12**) accepted carbon monoxide to generate the hexa-co-ordinated complex (**13**) which reproduces the split

† All the compounds described (save mesoporphyrin-II) are new and those marked with a dagger have been fully characterised spectroscopically (n.m.r., mass, and u.v.–visible spectroscopy) and by elemental analysis and/or accurate mass determination.

Table 1. ^{1}H N.m.r. signals (δ) from strapped porphyrins (250 MHz).

Substance	H-5, 10, 15, 20	Porphyrin-Me	CH_2*Me*	SCO*Me*	Central 6 CH_2's of strap
(**9**)[a]	10.09 (s, 1H) 10.05 (s, 3H)	3.65 (s, 6H) 3.62 (s, 3H) 3.61 (s, 3H)	1.88 (t, 6H)	2.17 (s, 3H)	Multiplets over range −0.3 to 1.8
(**11**)[b]	9.81 (s, 1H) 9.79 (s, 2H) 9.77 (s, 1H)	3.50 (s, 9H) 3.48 (s, 3H)	1.77 (t, 6H)	2.30 (s, 3H)	Multiplets over range 0.7 to −0.6
(**13**)[b]	9.39 (s, 1H) 9.31 (s, 1H) 9.27 (s, 1H) 9.25 (s, 1H)	3.38 (s, 3H) 3.37 (s, 3H) 3.34 (s, 6H)	1.72 (t, 3H) 1.65 (t, 3H)	—	Multiplets over range 0.5 to −3.9[c]

[a] Determined in $CHCl_3$. [b] Determined in CD_3SOCD_3. [c] This set includes the central –CH–CH_2–S– system.

Soret band in its u.v.–visible spectrum (Figure 1) so characteristic of the carbon monoxide complex of natural P-450.[6] The small absorption at 404 nm arises from a little material in which the thiolate ligand has been replaced by dimethyl sulphoxide (DMSO) [*cf.* chromophore (**11**)]. The distinctive resonances in the ^{1}H n.m.r. spectrum of the CO-complex (**13**) and from the macrocycles (**9**) and (**11**) are collected in Table 1.‡

Finally, the ^{13}CO-complex [as (**13**)] was prepared using 90 atom % ^{13}CO and the ^{13}C n.m.r. spectrum of this product in DMSO showed a single resonance at δ_C 196.8 p.p.m. This signal lies well upfield of the corresponding signals from haems X → Fe^{II}–^{13}C–O, where X is $-O^-$ or =N, which generally appear in the range δ_C 205—208 p.p.m.[7] This result should be compared with the similar upfield shifts (7—10 p.p.m.) shown by the natural system ^{13}CO–P-450$_{cam}$ (δ_C 200.3 p.p.m.)[7] and the recently prepared model with a thiolate 'tail' (δ_C 197.4 p.p.m.).[2a]

The synthesis is in progress of an analogue of complex (**13**) in which a cavity is to be formed over the CO-bearing face of the macrocycle by attaching a second strap incorporating anthracene (*cf.* ref. 3).

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‡ The high-field n.m.r. spectra of the strapped porphyrins show many interesting features which will be discussed in our full paper.

References

1 'Cytochrome P-450,' eds. R. Sato and T. Omura, Academic Press, New York, 1978; R. E. White and M. J. Coon, *Annu. Rev. Biochem.*, 1980, **49**, 315.
2 (a) T. G. Traylor, T. C. Mincey, and A. P. Berzinis, *J. Am. Chem. Soc.*, 1981, **103**, 7084; (b) P. Anzenbacher, Z. Sipal, B. Strauch, J. Twardowski, and L. M. Proniewicz, *ibid.*, p. 5928; (c) M. Schappacher, L. Ricard, R. Weiss, R. Montiel-Montoya, E. Bill, U. Gonser, and A. Trautwein, *ibid.*, p. 7646; (d) J. P. Collman and S. E. Groh, *ibid.*, 1982, **104**, 1391 and references in (a), (b), (c), and (d); (e) reviewed by C. K. Chang and D. Dolphin, *Bioorg. Chem.*, 1978, **4**, 37.
3 A. R. Battersby and A. D. Hamilton, *J. Chem. Soc., Chem. Commun.*, 1980, 117.
4 A. R. Battersby, D. G. Buckley, S. G. Hartley, and M. D. Turnbull, *J. Chem. Soc., Chem. Commun.*, 1976, 879.
5 T. Mincey and T. G. Traylor, *Bioinorg. Chem.*, 1978, **9**, 409.
6 Reviewed by M. J. Coon and R. E. White, in 'Metal Ion Activation of Oxygen,' ed. T. G. Spiro, Interscience, New York, 1980, p. 73.
7 A. P. Berzinis and T. G. Traylor, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 229.