Table II. Tlc Separation of Sterol Acetates ($AgNO_3$–Silica Gel)

A, benzene			B, solvent as indicated			
Band	R_f	Glc analyses rel retention times[a]	Solvent system[c]	Band	Glc analyses	RRT
1	Origin	*b*				
2	0.2–0.25	2 peaks	B–EA 9:1	2-1	Ergosta-5,7,24(28)-dien-3β-ol	2.2
				2-2	Ergosta-5,7,22,24(28)-tetraen-3β-ol	2.1
3	0.25–0.4	1 peak	B	3-1	Ergosterol	1.88
4	0.4–0.55	2 peaks	B–H 2:3 2×	4-1	Fecosterol	1.99
					Episterol	2.15
5	0.55–0.6	1 peak	B–H 4:1	5-1	Ergosta-7,22,24(28)-trien-3β-ol	2.05
6	0.6–0.65	1 peak	B–H 1:1	6-1	Zymosterol	1.64
7	0.65–0.85	4 peaks	B–H 1:5 2×	7-1[d]	Ergosta-7,22-dien-3β-ol	1.83
					Ergosta-8,22-dien-3β-ol	1.68
				7-2[d]	Ergost-7-en-3β-ol	2.17
					Ergost-8-en-3β-ol	1.97
8	0.85–front	Mixture of squalene, lanosterol, and 4,14-methylated sterols			Lanosterol	2.4
					4α-Methylzymosterol	1.82
					4,4-Dimethylzymosterol	2.28

[a] Relative retention times are relative to 3β-cholestanol on QF-1. [b] Complex mixture of polar material. [c] B, benzene; H, hexane; EA, ethyl acetate. [d] The components could be further separated by continuous tlc (24–48 hr) using 1:9 benzene–hexane as solvent.

amount of the particular sterol present in the yeast as determined by glpc above.

The incubation of labeled episterol with growing yeast is described in detail as an example.

Labeled episterol (28-^{14}C), 1.78×10^5 cpm, was incubated with growing yeast. After 7 hr the cells were collected by centrifugation and washed with phosphate buffer. Base hydrolysis and extraction gave the crude free sterol fraction which was dissolved in 50 ml of heptane. An aliquot of 0.5 ml was counted and had an activity of 940 ± 5 cpm. Since $100 \times 940 \pm 5$ cpm $= 9.4 \times 10^4 \pm 500$ cpm, the total incorporation = 52.6%. The free alcohols were acetylated and the total activity in the acetates was determined ($9.0 \times 10^4 \pm 500$ cpm).

The acetates were separated by tlc (five plates 20 × 20 cm), authentic sterol acetates were cochromatographed as markers, and the bands moving with ergost-7-enyl acetate, ergosta-7,22,24(28)-trienyl acetate, ergosta-5,7,22,24(28)-tetraenyl acetate, and ergosterol acetate were recovered.

To the recovered ergost-7-enyl acetate (1 mg) 30 mg of unlabeled sterol acetate was added and the mixture crystallized and rechromatographed two times (benzene–hexane, 1:1). The compound was not pure by glpc as judged by the presence of a small peak that had the retention time of the $\Delta^{7,22}$-diene acetate (12-Ac). The mixture was therefore submitted to continuous tlc for 48 hr (10% benzene in hexane). The recovered material was pure by glpc and after two crystallizations had a constant activity of 9.9 cpm/mg (based on carrier sterol) equivalent to 0.32% incorporation. Total activity recovered from the tlc band containing ergosta-7,22,24(28)-trienyl acetate was 1.60×10^4 cpm. Glpc showed small amounts of episterol to be present. Inactive material (100 mg) was added and the mixture recrystallized, rechromatographed on tlc, and recrystallized. The resulting material was pure by glpc and had an activity of 55.5 cpm/mg (based on carrier sterol) corresponding to 6.1% incorporation.

To the recovered ergosta-5,7,22,24(28)-tetraenyl acetate (~1 mg) 100 mg of unlabeled material was added. The material was purified two times by tlc and crystallized to constant activity: fifth recrystallization 48.5 cpm/mg, mp 142.5–144°. Based on added carrier sterol 4.85×10^3 cpm is equivalent to 6.1% incorporation. Unlabeled ergosterol acetate (100 mg) was added to the fraction containing this sterol (~1 mg) and the mixture purified by tlc on time and crystallization: fifth recrystallization 26.4 cpm/mg corresponding to an incorporation of 2.93%.

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Communications to the Editor

Binding of Dioxygen to Iron(II). Reversible Behavior in Solution

Sir:

The binding of dioxygen to hemoglobin has been the source of considerable interest and speculation.[1] In all known iron(II) complexes reaction with oxygen *in solution* is irreversible and leads, through autoxidation, to iron(III) species,[2] whereas hemoglobin exhibits the well-known reversible behavior with 1:1 stoichiometry, *per* iron atom.[3] The nature of iron–dioxygen complexes is also of relevence with regard to biological hydroxylation, and, in the hope of shedding light on these problems of reversibility and activation of dioxygen, we

(1) (a) L. Pauling, *Nature (London)*, 203, 182 (1964); (b) J. J. Weiss, *ibid.*, 203, 183 (1964); (c) J. S. Griffith, *Proc. Roy. Soc., Ser. A*, 235, 23 (1956); (d) J. Wittenberg, B. A. Wittenberg, J. Peisach, and W. E. Blumberg, *Proc. Nat. Acad. Sci. U. S.*, 67, 1846 (1970).

(2) (a) J. H. Wang, A. Nakahara, and E. B. Fleischer, *J. Amer. Chem. Soc.*, 80, 1109 (1958); (b) J. P. Collman and C. A. Reed, *ibid.*, 95, 2048 (1973).

(3) Several claims of reversible behavior in the oxygenation of iron(II) porphyrins in the solid state have been reported; *cf.* (a) A. H. Corwin and S. D. Bruck, *J. Amer. Chem. Soc.*, 80, 4736 (1958); (b) J. H. Wang, *ibid.*, 80, 3168 (1958).

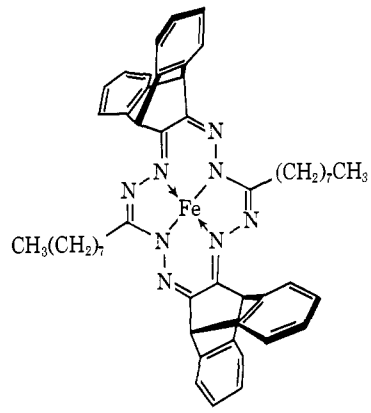
undertook to prepare and characterize discrete iron–dioxygen compounds. We report here the dependence of both reversibility and stoichiometry of oxygen binding by an iron(II) complex *in solution* on the degree of ligand encumbrance.

Studies on the autoxidation mechanism for iron(II) salts have suggested that an initial 1:1 binding of dioxygen by iron(II) (step a) is followed by a rapid bimolecular redox process (step b), which eventually leads irreversibly to iron(III) species,[4,5] eq 1. It ap-

$$\mathrm{Fe(II)} + \mathrm{O_2} \overset{a}{\rightleftharpoons} \mathrm{Fe^{II}O_2} + \mathrm{Fe(II)} \xrightarrow{b} \mathrm{Fe^{II}O\text{-}OFe^{II}} \dashrightarrow \mathrm{Fe(III)} \quad (1)$$

peared to us that if the second process (step b) were impeded by the molecular geometry of the iron(II) ligand it should be possible to obtain reversible behavior (step a). This situation might be approached by constructing a molecular cavity containing a suspended iron(II) atom, which would thereby approximate to each of the four porphyrin containing clefts of hemoglobin.[6] Since methods for rapid construction of such molecules are not yet developed, we used an open sided cavity, formed by 9,10-bridged-9,10-dihydroanthracenes, as in the compound **1**. This is a homolog of a compound previously reported by ourselves.[7] The parent macrocycle (mp 237–238°; λ_{max} ($CHCl_3$) 257 (ϵ 54,200), 339 mμ (ϵ 48,700) (*Anal.* Calcd for $C_{50}H_{56}N_8$: C, 78.1; H, 7.3; N, 14.6. Found: C, 78.0, H, 7.1, N 14.6) prepared by a modification of the previous procedure,[7] on condensation with freshly prepared, anhydrous, ferrous acetate in DMF at 100° in strictly anaerobic conditions,[8] gave complex **1**, as dark blue-black prisms recrystallized (DMF–acetonitrile) to mp 215° dec (vac. cap.); λ_{max} (DMF) 590, 625 mμ (*Anal.* Calcd for $C_{50}H_{54}N_8Fe$: C, 73.0; H, 6.6; N, 13.6; Fe, 9.2. Found: C, 73.4, H 7.0, N 13.5, Fe, 9.0). Accurate models[9] of rigid structure **1** indicate that the benzo substituents provide an open-sided cavity of depth 5 Å, as measured from the extremity of the van der Waals radii of the 4,5 hydrogens, as **2**. An analogous complex **3** was made from cyclohexane-1,2-dione by our described procedure,[7] λ_{max} (DMF) 590, 620 mμ (*Anal.* Calcd for $C_{30}H_{50}N_8Fe$: C, 62.3; H, 8.7; N, 19.4. Found: C, 62.1; H, 8.7; N, 18.9). This substance has a depth of 2.2 Å, measured from the extremities of the van der Waals radii of the quasiaxial hydrogens, as in **4**.

Reaction of **1** with oxygen at −78° in THF–dimethoxyethane (1:1 v/v) containing pyridine (4%) resulted in the formation of a bright, cherry red solution with the uptake of 1.0 mol of O_2 (±0.05).[10] To demonstrate the reversible nature of this binding we prepared samples of **1** in toluene–1% pyridine in a specially constructed spectral cell.[11] At −85° this solution showed λ_{max} 759 and 803 mμ,[12] and upon passage of dry oxygen over 4 min this absorption was replaced by λ_{max} 529 mμ. Three freeze–thawing cycles (−196 to −78°) reproduced essentially the original spectrum, measured as before. This process was carried out three more times with the results described in Figure 1.[13] These reversible spectra prove that complex **1** is regenerated on degassing, thus indicating a truly reversible uptake and release of dioxygen by an iron(II) complex. The corresponding iron(III) complex, prepared separately (acetate counterion), is characterized by different spectral properties, *i.e.*, λ_{max} 495 nm. Above −50° an irreversible degradation of the oxygenated complex **1** occurs to yield a substance of unknown structure.[14]



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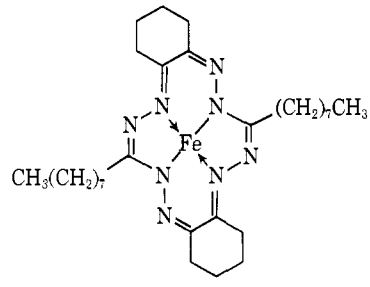

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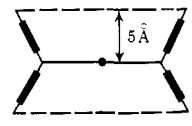

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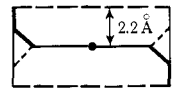

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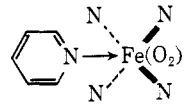

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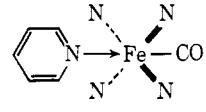

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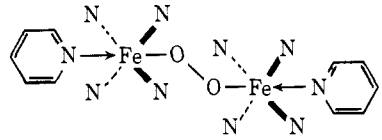

6

(4) (a) I. A. Cohen and W. S. Caughey, *Biochemistry*, 7, 636 (1968); (b) J. O. Alben, W. H. Fuchsman, C. A. Beaudreau, and W. S. Caughey, *ibid.*, 7, 624 (1968); (c) G. S. Hammond and C. H. S. Wu, *Advan. Chem. Ser.*, No. 77, 186 (1968).

(5) The factors possibly influencing reversibility in hemoglobin have been discussed in detail by J. H. Wang, *Accounts Chem. Res.*, 3, 91 (1970). The solubility properties of complex **1** have not enabled us to test the suggested influence of hydroxylic solvents, discussed by Wang.

(6) (a) M. F. Perutz, *Nature (London)*, **228**, 726 (1970); (b) J. C. Kendrew, *Science*, **139**, 1259 (1963).

(7) J. E. Baldwin, R. H. Holm, R. W. Harper, J. Huff, S. Koch, and T. J. Truex, *Inorg. Nuclear Chem. Lett.*, **8**, 393 (1972).

(8) All manipulations of these iron(II) complexes have of necessity to be carried out in strictly anaerobic conditions due to their *great* sensitivity to oxygen, both in solution and solid state.

(9) Dreiding stereomodels.

(10) Oxygen uptake was determined at constant pressure (atm) and temperature with a gas buret, using nitrogen filled ampoules of the complex.

(11) This cell was equipped with connections for oxygen and vacuum and could be maintained at temperatures down to −90°. Stoichiometric measurements[10] in the spectral solvent system were in complete accord with the earlier results.

(12) The long wave maxima of the nonoxygenated Fe(II) complexes are quite sensitive to solvent.

(13) The efficiency of reversibility, as judged from the optical densities, is >90% per cycle. The 1:1 complex is unstable > −50°. The absence of a clean isosbestic point is a consequence of a base line drift, resulting from a slight, time-dependent, fogging of the optics at the temperatures used.

(14) The structure of the irreversible product is under investigation.

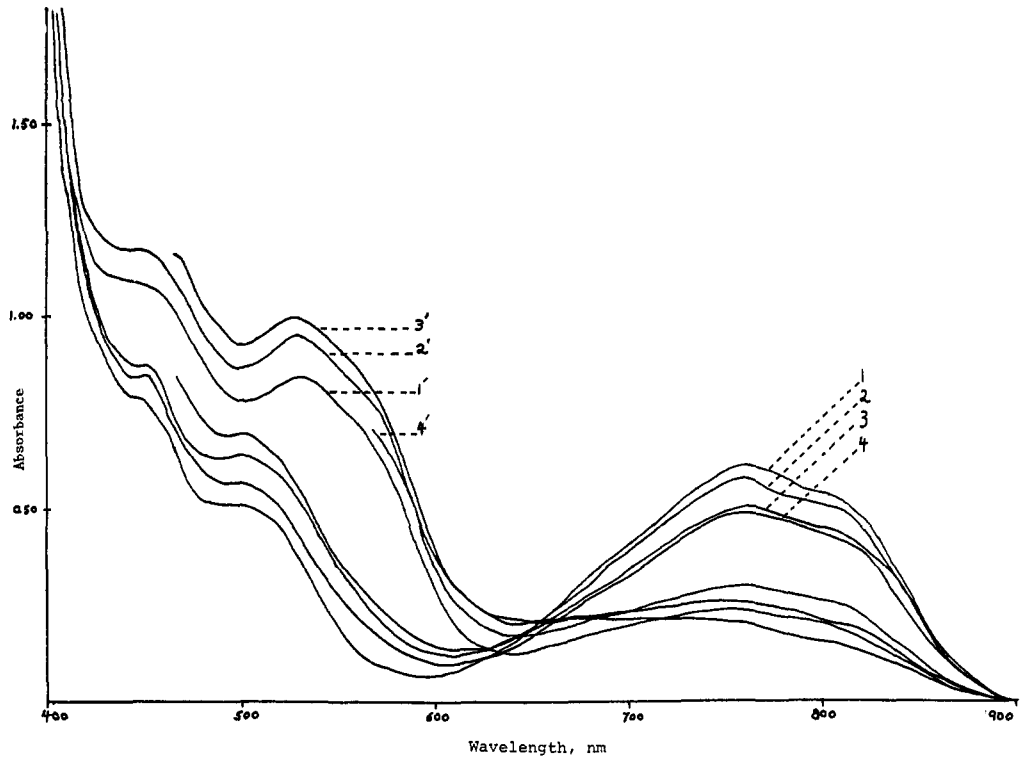

Figure 1. Reversible oxygenation of **1**. Curves 1–4 represent the reduced complex; curves 1′–4′ represent the oxygenated complex.

Pyridine *N*-oxide is not formed (glpc analysis) during this process.

In contrast the less hindered species **3** reacted with oxygen (toluene, pyridine (4%)) at −78° to give a red brown solution, λ_{max} 565 nm, with absorption of 0.5 mol of O_2 (±0.02). This uptake is apparently irreversible.

These experiments prove that the stoichiometry and reversibility of combination of these iron(II) complexes with oxygen are dependent upon the steric hindrance provided by the ligand. In the hindered case (**1**), a reversible 1:1 complex (**5**) has been formed, whereas in case **3** a complex of stoichiometry 2Fe(II) per O_2, **6**, was obtained.[15] Complex **1** combined smoothly with carbon monoxide to give a 1:1 complex (**7**), λ_{max} (DMF) 505 mμ.

The formation of 1:1 and 2:1 complexes of various Co(II) species with dioxygen is well known,[16] but these differ greatly from the iron species in stability and consequently do not require steric encumbrance in the ligand. However, an X-ray analysis of a 2:1 cobalt–dioxygen complex indicates a Co–Co distance of 4.5 Å.[17] A similar Fe–Fe distance would be available at the closest approach of two molecules of complex **3**, as in structure **6**. This fact offers an explanation for the sterically dependent phenomena described here.

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(15) We suggest these formulations, **5**–**7**, without implication of the nature of binding of oxygen, as the most likely ones on the basis of present evidence.

(16) (a) D. Diemente, B. M. Hoffman, and F. Basolo, *Chem. Commun.*, 467 (1970); (b) J. H. Bayston, N. King, F. D. Looney, and M. E. Winfield, *J. Amer. Chem. Soc.*, **91**, 2775 (1969); (c) C. Floriani and F. Calderazzo, *J. Chem. Soc. A*, 946 (1969).

(17) M. Calligaris, G. Nardin, and L. Randaccio, *Chem. Commun.*, 763 (1969).

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Stereochemistry of Methyl Group Insertion in Corrinoid Biosynthesis.[1] Determination of Carbon Isotope Chirality by ^{13}C Nuclear Magnetic Resonance[2]

Sir:

Recent experiments with whole cells of *Propionibacterium shermanii*[3] implicating uroporphyrinogen III (**2**, urogen III) as a precursor for vitamin B_{12} (**3a**, cyanocobalamin) have placed restrictions on the timing of the methylation process in corrin biosynthesis. Before developing further mechanistic proposals for the latter sequence, which appears to be controlled by both steric and electronic consequences of methyl group insertion *via* *S*-adenosylmethionine (leading to α orientation in rings A and B and β orientation in ring D), resolution of the problem of the stereochemistry of methylation at C-12 in ring C became necessary. Thus, although it has been rigorously demonstrated[4] that one

(1) Presented in part at the Peter A. Leermakers Symposium, Wesleyan University, Nov 29, 1972, and available on a "Science Symposia on Tape" from the American Chemical Society.

(2) Carbon-13 Fourier Transform Nmr, Part VII. Part VI: H. H. Wasserman, R. J. Sykes, P. Peverada, C. K Shaw, R. J. Cushley, and S. R. Lipsky, *J. Amer. Chem. Soc.*, in press.

(3) A. I. Scott, C. A. Townsend, K. Okada, M. Kajiwara, and R. J. Cushley, *ibid.*, **94**, 8269 (1972); A. I. Scott, C. A. Townsend, K. Okada, and M. Kajiwara, *Trans. N. Y. Acad. Sci.*, **35**, 72 (1973).

(4) R. C. Bray and D. Shemin, *J. Biol. Chem.*, **238**, 1501 (1963).