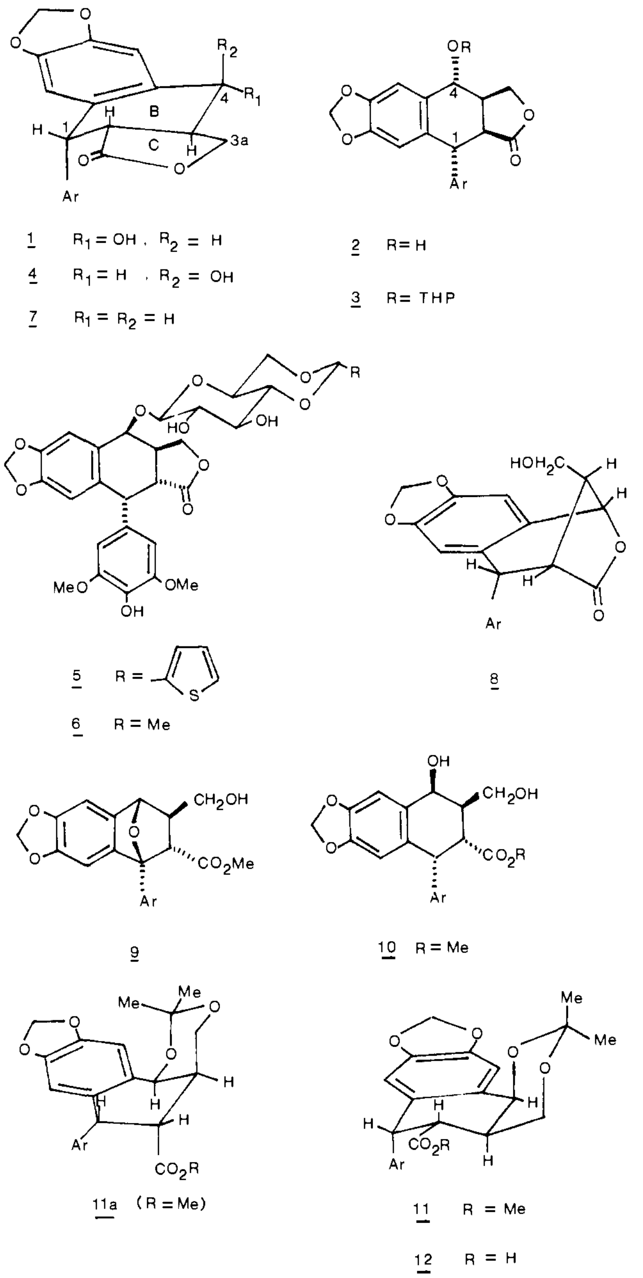
Chart I



Ar = 3,4,5-trimethoxyphenyl

The entire sequence from bromopiperonal can be completed in 2 weeks and requires no chromatography except for a filtration column to remove dicyclohexylurea after the DCC lactonization. Practical stereocontrolled syntheses of **1** (9.4%, 12 steps[16]), **4** (6%, 11 steps), and **8** (14.9%, 10 steps) have thus been achieved.

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[15] **4**; 85% yield; m.p. 211 °C; $\nu_{C=O}^{CHCl_3}$ 1780 cm^{-1}. The ^{1}H NMR spectrum of **4** was identical with a published trace (Brewer, C. F.; Loike, J. D.; Horowitz, S. B.; Sternlicht, H.; Gensler, W. J. *J. Med. Chem.* **1979**, *22*, 215).

[16] The figure of 63% is based on the crude yield (78%) of podophyllic acid obtained from the saponification of **8** by the previous authors. Purification of the crude product provided podophyllic acid in only 29% yield[14] which reduces the overall yield for the two steps to 24%. Our overall yield of 9.4% for **1** is based on the higher figure.

Photoassisted Reduction of Molecular Oxygen to Hydrogen Peroxide Catalyzed by Oxoalkoxomolybdenum(V) Porphyrin

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In the last few years much attention has been focused on the photochemistry of metalloporphyrins as these complexes exhibit very intense absorptions in the visible region.[1] This makes them choice candidates in the design of light-harvesting systems for solar energy conversion. However, very few examples of the photochemistry of porphyrins with a redox-active central metal have been described.[2] Recently we reported on the photolysis of a diperoxomolybdenum(VI) porphyrin $(O_2)_2Mo(TPP)$ which affords the related *cis*-dioxo complex $O_2Mo(TPP)$.[3-5] The paucity of such a photoejection of a dioxygen ligand from a transition-metal complex[6,7] led us to study the photochemical behavior of molybdenum porphyrins containing Mo–O bonds.[8]

When $O{=}Mo^{V}(TPP){-}OCH_3$[9] (**1**) was aerobically irradiated (100-W tungsten lamp) in a benzene solution containing 5% v/v methanol, a clean evolution of the UV–visible spectrum was observed as shown in Figure 1, affording a new absorbance at λ 431 nm in the Soret region, characteristic of $O{=}Mo^{IV}(TPP)$ (**2**).[10] When this solution was left in the dark, the spectra of **1** was fully restored. The dependence of the wavelength of irradiation on the reaction was examined by using a monochromatic light source.[11] No noticeable decomposition of **1** was observed when a benzene solution (1.4×10^{-6} M) was irradiated in a 5-cm pathlength cell at λ 620 or 575 nm near the maximum of absorption, respectively, of the α and β bands. However a rapid evolution to **2** was obtained when this solution was irradiated in the Soret region. The quantum yield for the reaction, determined by using the ferrioxalate ac-

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[3] Abbreviations used: *meso*-tetraphenylporphyrinato, TPP; *meso*-tetra-(*p*-tolyl)porphyrinato, TTP; octaethylporphyrinato, OEP; electron paramagnetic resonance, EPR.

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[10] $O{=}Mo^{IV}(TPP)$ was prepared as described for $O{=}Mo^{IV}(TTP)$: Diebold, T.; Chevrier, B.; Weiss, R. *Inorg. Chem.* **1979**, *18*, 1193–1200.

[11] 150-W xenon lamp OSRAM XBO and Bausch and Lomb monochromator were used. Slides were adjusted to provide a spectral band width of about 10 nm. Filters M.T.O. J 351, Kodak W 4, and Kodak W 25 were, respectively, used for irradiations at 454, 575, and 620 nm.

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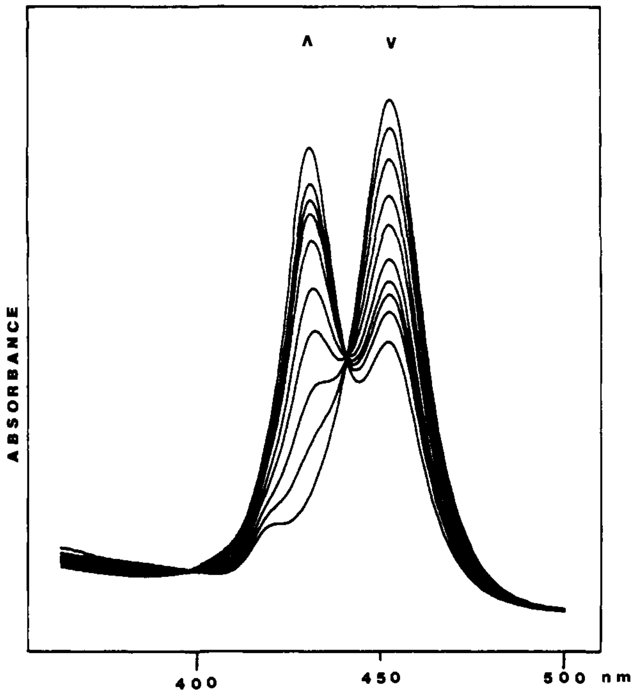


Figure 1. Evolution of the visible spectra of a solution of O=Mo(TPP)—OCH_3 in benzene–methanol mixture (95:5) in the presence of air. Arrow heads indicate the variation of absorbance.

tinometer method,[12] was 1.5×10^{-2}. No fluorescence was detected. Formation of the oxomolybdenum(IV) porphyrin **2** by photoinduced homolysis of the Mo–OCH_3 bond was confirmed by EPR spectroscopy and spin-trapping experiments.

$$\mathrm{O{=}Mo^{V}(TPP){-}OCH_3} \xrightarrow{h\nu} \mathrm{O{=}Mo^{IV}(TPP)} + \cdot\mathrm{OCH_3} \quad (1)$$

When an oxygen-free benzene solution of **1** was irradiated[13] directly in the cavity of an EPR spectrometer, the intensity of the characteristic nine-line spectra of **1** slowly diminished with time but again was fully restored when the cell was open to air and one drop of methanol was added, confirming the formation of a diamagnetic molybdoporphyrin complex upon irradiation. When the same experiment was repeated in the presence of α-phenyl-N-*tert*butylnitrone, a new signal ($g = 2.0062$, $A_N = 12.75$ G, $A_H = 1.78$ G) attributed to the methoxyl radical–nitrone adduct[14] appeared, concomitant with the bleaching of the spectrum of **1**. The formation of a radical species was also demonstrated by the polymerization of acrylonitrile[15] by irradiation of a benzene solution (20% v/v) containing ca. 0.1% of **1**, which affords a cream insoluble material (ν_{CN} 2343 cm^{-1}).

The reoxidation of O=Mo^{IV}(TPP) (**2**) in a benzene–methanol mixture was monitored by UV–visible spectrometry. Figure 2 shows the evolution of the concentration of **2** against time, which follows strict second-order kinetics. This second-order dependence on molybdenum suggests a two-electron reduction of molecular oxygen to hydrogen peroxide which was actually identified in separate experiments.

$$\mathrm{2O{=}Mo^{IV}(TPP)} + \mathrm{O_2} \xrightarrow{\mathrm{CH_3OH}} \mathrm{2O{=}Mo^{V}(TPP){-}OCH_3} + \mathrm{H_2O_2} \quad (2)$$

In order to evaluate the efficiency and possible uses of the photoreduction of **1**, a catalytic cycle was devised by coupling the reactions described in reactions 1 and 2. When a 1.9×10^{-5} M solution of **1** in dry benzene–methanol mixture (90:10) was exposed to sun light by using a watercooled jacketed Pyrex reactor over a period of 4 h, hydrogen peroxide could be detected by iodometric titration.[16] Up to 56 mol of hydrogen peroxide have been obtained per mole of **1** per hour giving a final concentration of 4.3×10^{-3} M. The fate of the methoxyl radical was not studied as it was reported to afford mainly formaldehyde under similar conditions.[17] In fact, when cyclohexanol was used instead of methanol, cyclohexanone was identified by gas chromatography, but the reaction was much slower (4–5 rotations per h) probably due to the larger steric hindrance of the alcohol. The overall reaction which is the formation of hydrogen peroxide from molecular oxygen using methanol as reducing agent is exergonic as may be estimated from the thermodynamic standard data.[18]

$$\mathrm{CH_3OH} + \mathrm{O_2} \rightarrow \mathrm{CH_2O} + \mathrm{H_2O_2} \quad (3)$$

In this catalytic cycle, solar energy is thus only used to furnish the required activation energy for the reaction. However, the photochemical step (reaction 1) is probably largely endergonic. The redox potential for the reduction of **1** has been reported in CH_2Cl_2 solution to be −0.74 V vs. SCE,[19] giving a free energy change of

$$\mathrm{O{=}Mo^{V}(TPP){-}OCH_3} + 1e^- \rightarrow \mathrm{O{=}Mo^{IV}(TPP)} + \mathrm{CH_3O^-} \quad (4)$$

about + 11 kcal/mol and the electron affinity of the methoxyl radical, EA, equal to 1.8 eV,[20] i.e., $\Delta H = -42$ kcal/mol. If the contribution of solvation effects and the entropic term in reaction 5 is neglected, the free energy change of reaction 1 (reaction 4 − reaction 1) is largely positive.

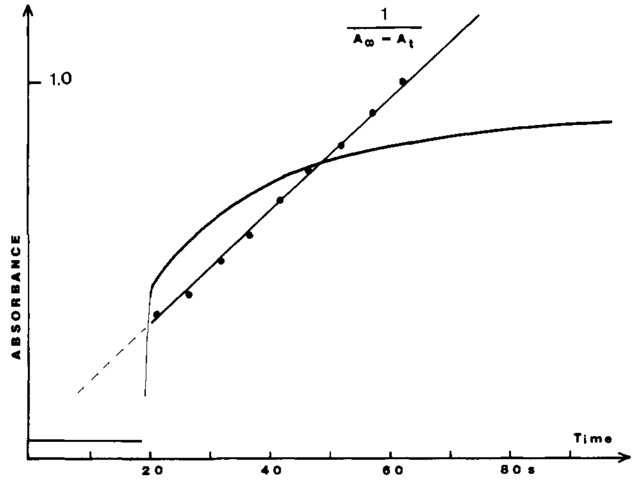
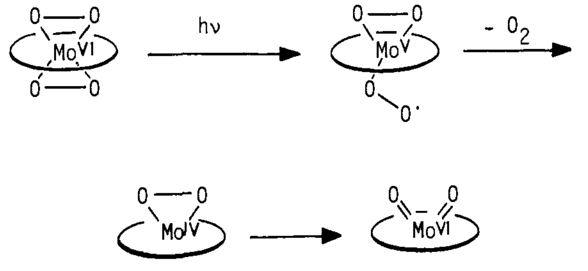


Figure 2. Time-dependent evolution of the absorbance at 619.5 nm of a solution of O=Mo(TPP) in benzene containing 1% of methanol after exposure to air. A delay of ca. 20 s was needed before recording to insure a correct mixing.

Scheme I



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$$CH_3O\cdot + 1e^- \rightarrow CH_3O^- \qquad (5)$$

The easy photoreduction that we observed for $O{=}Mo^{V}(TPP){-}OCH_3$ may account for the spectral change caused by laser irradiation of $O{=}Mo^{V}(OEP){-}OCH_3$ reported by Ohta et al.[21] during the measurement of resonance Raman spectra in KBr pellets. The appearance of a new band at 956 cm^{-1} is in close agreement with the value of the Mo—O stretch observed in infrared spectroscopy at 965 cm^{-1} for $O{-}Mo^{IV}(OEP)$.[22]

This also gives a reasonable pathway for the photodecomposition of the diperoxomolybdenum(VI) porphyrin $(O_2)_2Mo^{VI}(TPP)$. We can assume, as favored by extended Hückel calculations,[23] that the first step is the homolysis of a Mo-O bond as shown in Scheme I, affording formally a peroxo-superoxomolybdenum(V) complex. This intermediate should be very unstable as there is no example of a fully characterized peroxo complex of d^n, n odd, transition metal[24] and recently an oxomolybdenum(V) porphyrin complex has been reported to be reduced by superoxide ion.[25] Next step will be the departure of molecular oxygen providing a peroxomolybdenum(IV) complex which will give the *cis*-dioxomolybdenum(VI) porphyrin after a very thermodynamically favorable internal redox reaction.[23]

These results clearly establish the ability of molybdenum porphyrins to harvest solar energy to produce reactive intermediates by redox processes.

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Semisynthetic Enzymes: Synthesis of a New Flavopapain with High Catalytic Efficiency

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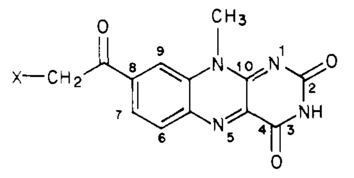
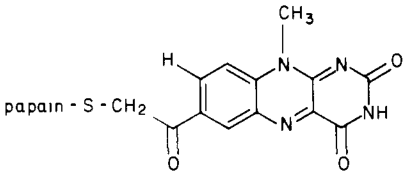
Flavopapain **1**, derived from alkylation of the active site cysteine-25 of papain [EC 3.4.4.10] with the reactive flavin analogue **2**, is a highly efficient semisynthetic enzyme, whose catalytic activity surpasses that of our best previously described flavopapain **4**[1-3] by over an order of magnitude and begins to approach the activity exhibited by all but the most efficient flavin-containing oxidoreductases. We feel that this represents a significant step toward the production of enzymelike molecules by a process that we call "chemical mutation". This is an approach to the design of catalysts which relies on the combination of an existing protein-binding site with a chemically reactive coenzyme analogue, thus exploiting the binding specificity of the protein but expressing the characteristic chemical reactivity of the covalently attached coenzyme.

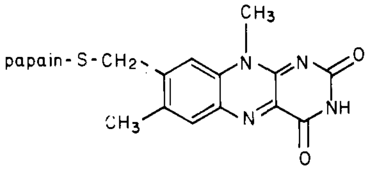


1, X = papain-S-
2, X = Br
3, X = H



4

It has been demonstrated that flavopapain **4** is an effective catalyst for the oxidation of N^1-alkyl-1,4-dihydronicotinamides by O_2.[1-3] Rates of the enzymatic oxidation exceed those of the flavin prosthetic group by an order of magnitude. More importantly, catalysis by the semisynthetic enzyme **4** exhibited substrate saturation kinetics, as would be expected for an enzymatic reaction involving the obligatory participation of a binding site. In contrast, oxidation reactions catalyzed by flavopapain **5** exhibited modest rate enhancements of the order of about 3-fold and did not exhibit saturation kinetics.



5

The difference between flavopapains **4** and **5** had been anticipated on the basis of model building, assuming that the carbonyl oxygen of **4** could hydrogen bond to the peptide backbone of papain, thus positioning the flavin within the hydrophobic binding groove of the enzyme.[4] No such interaction is possible in the case of **5**, and hence no comparable positioning of the flavin prosthetic group seems likely in this case.

Further model building, this time using structurally similar 8-acetyl-10-methylisoalloxazine attached as in **1**, with the acetyl carbonyl constrained to participate in a scheme of hydrogen bonding identical with that postulated to occur in the case of **4**, led to the prediction that N^1-benzyl-1,4-dihydronicotinamide (N^1-BzNH) could fit snugly within the binding pocket of the enzyme, in close proximity to the flavin. Hence, the prediction was made that flavopapain **1** should be a good catalyst for the oxidation of this substrate and fall into that group of semisynthetic enzymes which exhibits saturation kinetics and relatively high catalytic rate enhancements.

In order to test this prediction, a synthesis of the requisite 8-acetyl-10-methylisoalloxazine **3** had to be devised. This was achieved starting with the well-characterized-3-amino-4-nitroacetophenone **6**[5] as shown in Scheme I.

Treatment of amine **6** with trifluoroacetic anhydride in a solvent of trifluoroacetic acid at 0 °C for 20 min led to the isolation of 3-(trifluoroacetamido)-4-nitroacetophenone as short yellow needles

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