A Novel Heterocyclic *N*-Oxide, Pyrimido[5,4-*g*]pteridinetetrone 5-Oxide, with Multifunctional Photo-oxidative Properties. Efficient Agents for Photo-oxygenation and -dehydrogenation, and for Photochemical Generation of Hydroxyl Radicals.

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Abstract : During our search for heterocyclic *N*-oxides possessing high photo-oxidation capacity, pyrimido[5,4-*g*]pteridinetetrone 5-oxides were discovered to fulfill this role. Chemical and physico-chemical studies clearly indicated that the *N*-oxides behave as an electron acceptor to cause efficiently photo-oxygenation, photo-dehydrogenation and photochemical generation of hydroxyl radicals, depending on the nature of the substrates and solvents employed. Thus, the *N*-oxides behave like oxygen under photochemical conditions and their multi-photooxidative functions mimic formally those of iron oxenoid.

In this review, synthesis, photochemical properties and application of the novel *N*-oxides are described and discussed in the light of impetus for future investigation.

Key phrases : pyrimido[5,4-*g*]pteridinetetrone 5-oxide ; photo-oxygenation ; photo-dehydrogenation; hydroxyl radical generation ; single electron transfer ; DNA cleaving agent.

Introduction

The photochemistry of heterocyclic *N*-oxides has been extensively studied, but some details remain equivocal.[1] The known photochemical reactions of the heterocyclic *N*-oxides are classified as intramolecular rearrangements of the *N*-oxide function or intermolecular oxygen-atom transfer to co-substrates (deoxygenation). Although the latter can be considered to be a simple chemical model for biological mono-oxygenations, the understanding of the mechanism for this photoreaction is less advanced than for the former reaction, because it is usually not the predominant process in the photochemistry of the heterocyclic *N*-oxides.

Two interpretations have been given for the photochemical oxygen atom transfer reaction of the *N*-oxides ; (i) the interception of an oxygen atom generated in the triplet-excited state by substrates (the oxene mechanism) ; (ii) the oxygenation of substrates by the oxaziridine intermediate which is formed in the singlet-excited state but to a large extent undergoes intramolecular rear-

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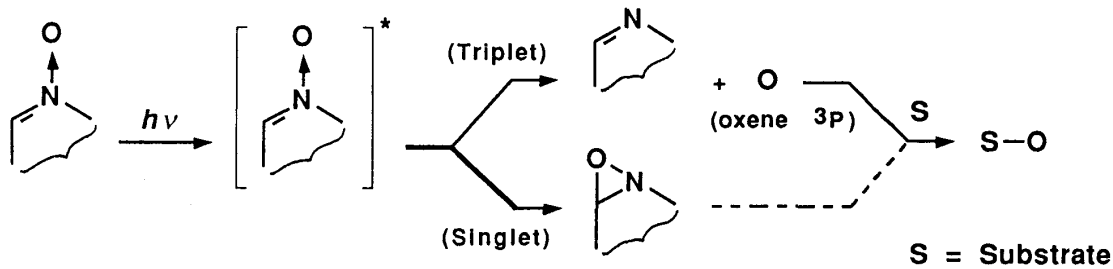
rangements (see Scheme 1). The former interpretation has been generally accepted for photo-oxygenation by the heterocyclic N-oxides.



Scheme 1

During our search for heterocyclic *N*-oxides which could serve as an effective oxygen-atom transfer agent without accompanying intramolecular rearrangements under photochemical conditions, we found that 1,3,7,9-tetrabutylpyrimido[5,4-*g*]pteridine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)tetrone 5-oxide (PPO) is suitable for this purpose.

We also proved that PPO functions efficiently as an agent for dehydrogenation depending on the nature of substrates. These photo-oxidations by PPO in organic solvents (*e.g.*, MeCN) were elucidated in most cases to involve a single electron transfer (SET) in the excited state, which are unique among the heterocyclic *N*-oxides investigated so far. While the detailed mechanisms for oxidation of various substrates with the oxidizing species ($Fe^{V}=O$) in cytochrome *P*-450 enzymes are still being debated,[2] the photo-oxidation by PPO can be formally considered to be a reaction mimic for the hemin-catalyzed oxidations.

Photolysis of hydrophilic 1,9-di (methoxymethyl) -3,7-dimethylpyrimido [5,4-*g*] pteridine-2,4,6,8 (1*H*,3*H*,7*H*,9*H*)-tetrone 5-oxide (PPOM) in water conveniently generated clean hydroxyl (OH) radicals. This new OH radical generator will hopefully provide impetus for further investigations of the OH radical chemistry involving the design of a DNA cleaving agent.

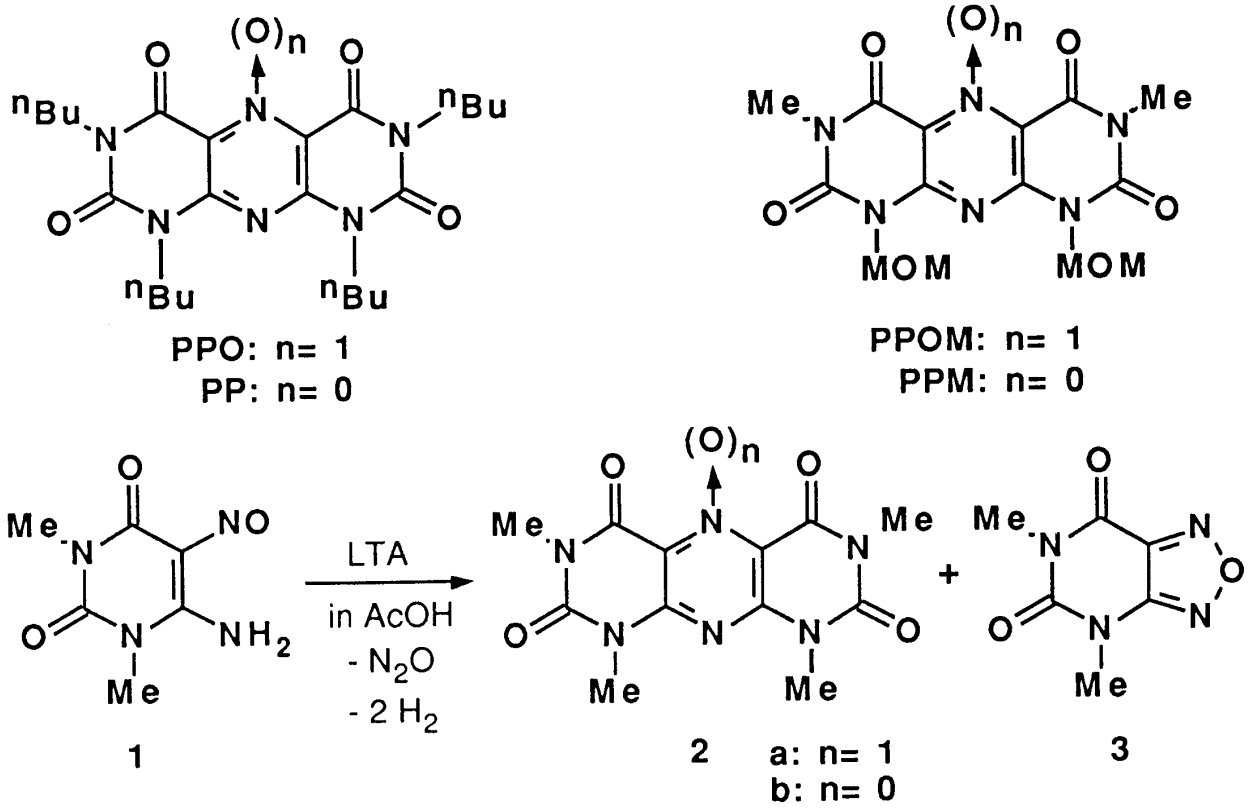
This account describes the remarkable multi-functional photochemical properties of PPO and PPOM, and their practical applications.

Syntheses and Structures of 1,3,7,9-Tetra-*n*-butyl- and 1,9-Di(methoxymethyl)-3,7-dimethyl-pyrimido[5,4-*g*]pteridinetetrone 5-Oxides, PPO and PPOM

In 1972, the author, together with Taylor and McKillop, reported that 6-amino-1,3-dimethyl-5-nitrosouracil **1** undergoes smooth oxidation with lead tetraacetate (LTA) in acetic acid to give dimeric *N*-oxide **2a** along with a small amount of 4,6-dimethylfurazano[3,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione **3**.[3a]

The structure of **2a** was assigned on the basis of degradation studies and was confirmed both by its independent synthesis[4] and X-ray crystallographic analysis.[5]

Since it was not possible to form the 5-oxide **2a** by peracid oxidation of the parent pyrimido-



Scheme 2

[5,4-*g*]pteridinetetrone **2b**, the oxidative dimerization of 1 with LTA remains the only viable method for the preparation of this intriguing *N*-oxide. This type of oxidative dimerization occurred in various 1,3-disubstituted uracils (cf. **1**), but not when 1,3-unsubstituted or 1- or 3-monosubstituted uracils were employed in place of **1**.[3b] Thus, PPO[3] and PPOM[6] were prepared with ease by analogous oxidation of the corresponding 6-amino-5-nitrosouracils with LTA in 80% and 50% yields, respectively.

^{18}O-labeled *N*-oxides, PP^{18}O and PP^{18}OM, were obtained as follows : treatment of the corresponding 1,3-disubstituted 6-aminouracils with nitrosonium tetrafluoroborate in MeCN containing ^{18}O-labeled water gave 1,3-disubstituted 5-amino-^{18}O-labeled 5-nitrosouracils (cf. **1** with $N^{18}O$ at the 5-position). Subsequent oxidation of the ^{18}O-labeled nitroso derivatives with LTA resulted in the formation of PP^{18}O[3b] and PP^{18}OM[6] which certainly contain ^{18}O in the *N*(5)-oxide grouping (^{18}O-content = *ca.* 56%).

ESR studies and a number of chemical observations suggested a novel reaction sequence involving oxidative dimerization of **1** followed by intramolecular cyclization, oxidation, and homolytic elimination of nitrous oxide.[3b]

Physicochemical Properties of PPO and PPOM

PPO (mp 158 °C) is soluble in various organic solvents, *e.g.*, MeCN and cyclohexane, in contrast with hardly soluble **2a**. Thus, PPO was chosen as a favorable specimen for the investigation

on the photochemistry in the organic solvents. On the other hand, PPOM (mp 265 °C) is advantageous for photochemical studies in aqueous solutions because of its high solubility in water.

PPO shows UV (in MeCN): 370 (ε =2.2x 10^4) nm; Fl (in MeCN): 394 (λ ex : 370) nm, t_S : 2.95 ns[7] : $E_{1/2}^{red}$ (in MeCN) : -0.97 V *vs* SCE.[8a]

The corresponding anion radical generated during controlled potential electrolysis of PPO in dry MeCN showed a hyperfine splitting signal with $a_{N(5)}$= 14.05 G (1G= 10^{-4} T) in its ESR spectrum. HMO calculation for the anion radical suggested the high localization of spin density in the *N*(5)-oxide.[5]

The relevant MO character and the change of the *N*(5)-*O* bond order in the lowest π-π^* excited singlet state calculated by the INDO/S-CI method suggested that PPO is characteristic in comparison with other common *N*-oxides, accommodating its unique photochemical properties.[9]

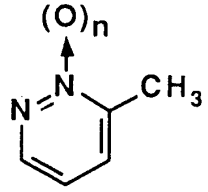
These diverse properties are probably the consequence of an exceptionally weak *N*(5)-*O* bond and suppression of intramolecular rearrangements of the *N*(5)-oxide in the excited state due to its two flanking carbonyl groups and its attachment to the strongly electron-deficient uracil rings. The X-ray crystallography of **2a** showed that although the *N*(5)-*O* bond is in coplanar with the pyrimido[5,4-*g*]pteridine ring, the adjacent carbonyl groups are forced to bend slightly outward.[5]

PPOM shows UV (in water) : 360 (ε =1.5 x 10^4) nm and E_P^{red} (in water) : - 0.70 V vs. SCE.

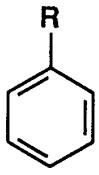
Photochemical Oxygenation by PPO

1. Photo-oxygenation of Benzene Derivatives[8]

Photo-oxygenation of benzene (**4a**), toluene (**4b**), and anisole (**4c**) by PPO was compared with that by 3-methylpyridazine 2-oxide (MPO),[8a] which has been proposed to oxygenate substrates such as alkanes and aromatic compounds by the oxene mechanism.[10]



MPO: n = 1
MP: n = 0



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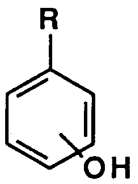

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a: R = H
b: R = CH_3
c: R = OCH_3
d: R = $N(CH_3)_2$
e: R = SCH_3

Scheme 3

The distinct differences in the photo-oxygenation of **4a-c** by PPO and MPO under analogous conditions (**4a-c** : 2.0 M, *N*-oxide: 5.0 mM in MeCN, external irradiation with a 400W high-pressure Hg lamp, Pyrex filter, under Argon) are as follows.[8a]

i) PPO oxygenated **4a** more slowly than did MPO. The deoxygenation of PPO to PP, however, occurs quantitatively without any accompanying intramolecular rearrangement of its *N*-oxide group taking place. When the photoreaction was carried out with irradiation for a short time (less than 1 h) so that a significant amount of PPO was recovered, phenol **5a** was obtained

quantitatively based on PPO consumed. Longer irradiation times led to lower yields of **5a** (*e.g.* 2.5 h, 50%), indicating clearly the occurrence of further photoreactions. An independent experiment demonstrated that irradiation of a mixture of **5a** and PPO under analogous conditions smoothly produced *o*- and *p*-catechols.[8c] (*vide infra*) In the case of MPO, **5a** was obtained in 34% yield from **4a** after irradiation for 20 min, accompanying with significant amounts of rearrangement products. Only slight amounts of 3-methylpyridazine, MP, was detected in agreement with previous observations.[10]

ii) The following product distributions in the photo-oxygenations were observed: the photo-oxygenation of **4b,c** by PPO resulted in the formation of phenols **5b,c** as a mixture of *o*- and *p*-isomers, whereas MPO gave **5b,c** as a mixture of *o*-, *m*-, and *p*- isomers. In the cases of **4b,c**, PPO gave phenol **5a** from **4c**, and benzyl alcohol, benzaldehyde, and 1,2-diphenylethane from **4b**, which are reaction products in the respective substituents, as minor products. These products were not detected in the photo-oxygenation by MPO.

iii) PPO was stable in MeCN on irradiation, and the consumption rate of PPO was clearly dependent on the concentration of the substrate **4c**, indicating an appreciable interaction between PPO and **4c** in the photo-oxygenation. In sharp contrast to the case of PPO, MPO was unstable in MeCN on irradiation and the consumption rate of MPO was not affected by the presence of **4c**, indicating that MPO liberates an atomic oxygen directly from the excited MPO and oxygenates **4c** as previously reported.[10c]

The above experimental facts evidently show that PPO photochemically oxygenates **4a-c** with high efficiency and in a different reaction mode from that of MPO.

The quantitative transfer of an oxygen-atom from PPO to the substrates was proved by experiments using PP^{18}O. Wavelength-dependence experiments showed that the consumption of PPO in the photoreaction with **4c** occurs most efficiently on irradiation at *ca.* 365 nm, which is near the longest UV absorption band of PPO and excites PPO exclusively.

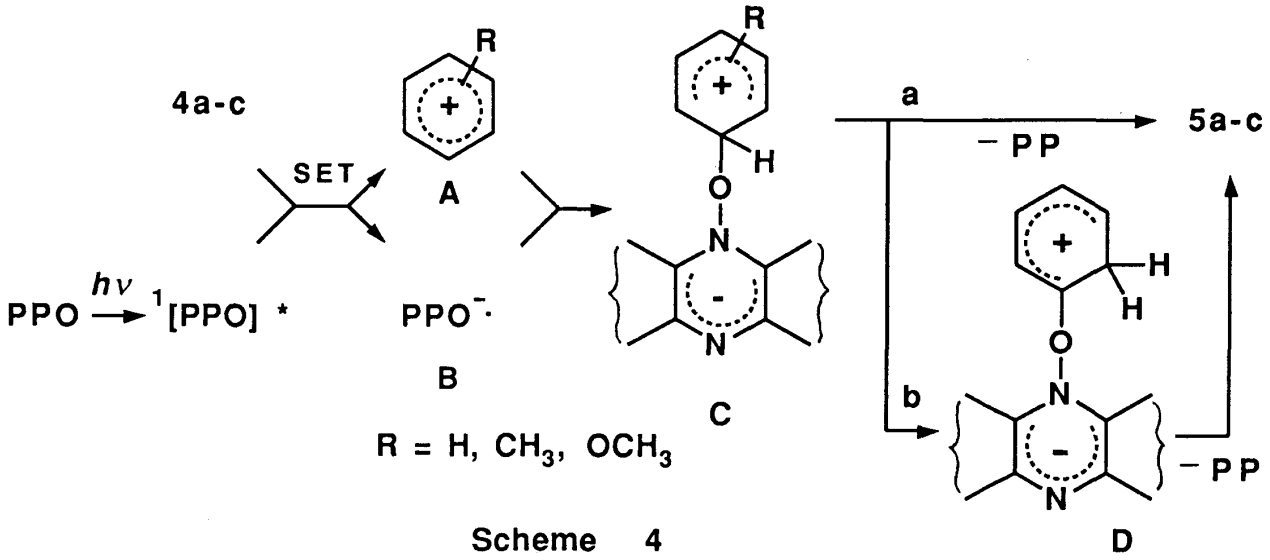
The fluorescence of PPO in dry MeCN was effectively quenched by the addition of **4c**. The quenching rate constant (k_q) was estimated to be 2.1 x $10^9 dm^3 mol^{-1} s^{-1}$. The photo-oxygenation of **4c** by PPO was not affected by the addition of a triplet sensitizer such as acetone, acetophenone, or benzophenone and of a triplet quencher such as biacetyl.

These results indicate that singlet-excited PPO is responsible for the present photo-oxygenations. The addition of tetracyanoethylene or tetracyanoquinodimethane, a strong electron acceptor, to the reaction medium containing **4c** and PPO markedly inhibited the formation of **5c** even at low concentrations. According to the Rehm-Weller treatment,[11] the free energy changes (ΔG_{et}) for the SET from **4a-c** to the singlet-excited PPO were roughly calculated to be - 0.6 kcal mol^{-1} for **4a**, - 7.5 for **4b**, and - 12.1 for **4c**, which are linearly related to the relative consumption rates of PPO in the respective reactions.

The above results well accommodate the involvement of the SET from **4a-c** to the singlet-excited

PPO in the initial stage of the photoreaction.

Taking above facts into consideration, the reaction sequence in Scheme 4 is a reasonable rationalization of the photo-oxygenation of the benzene derivatives **4a-c** by PPO.[8a]



Scheme 4

The photo-oxygenation of the benzene ring by PPO proceeds *via* the formation of a zwitterionic intermediate **C** by coupling of the radical ions, **A** and **B**, generated as a result of the SET from **4a-c** to the singlet-excited PPO. The photochemical formation of phenol **5a** from **4a** by PPO was efficiently accelerated by the addition of trifluoroacetic acid (TFA). The catalytic role of TFA is most likely explained in terms of suppression of the back electron-transfer taking place between the radical ions, **A** and **B**, and stabilization of the zwitterionic intermediate **C** by protonation.[12]

Fragmentation of the intermediate **C** gives phenols **5a-c** and PP accompanied by direct proton loss (route *a*). 1,2-Hydrogen shift, namely the 'NIH shift', in **C** leads to a transient intermediate **D** which collapses to give **5a-c** and PP (route *b*). In fact, the photochemical oxygenation of [4-^2H]-toluene and [4-^2H]-anisole by PPO resulted in the formation of *p*-cresol and *p*-methoxyphenol with deuterium content of 38% and 18%, respectively.

In the case of **4b**, the proton transfer from the methyl group in **A** (R=Me) to **B**[13] generates a benzyl radical and an PPO-nitroxyl radical. Self-coupling of the benzyl radical produces 1,2-diphenylethane. Coupling of both radicals could give benzyl alcohol and PP *via* fragmentation of the resulting intermediate. The alcohol thus formed can be dehydrogenated by the singlet-excited PPO to give benzaldehyde *via* the SET process (*vide infra*).

In the case of **4c**, proton abstraction from the cation radical **A** (R=OMe) by **B** generates a phenoxymethyl radical and nitroxyl radical (see **G** in Scheme 9) which give a coupling intermediate. Fragmentation of the intermediate could give rise to **5a**, PP, and formaldehyde. This type of oxygenative demethylation was observed to occur with ease in the photoreaction of dimethylaniline **4d** with PPO.[8d] In this case, however, the initial SET from **4d** to PPO can take place *via* an excited CT-complex, because excitation of the **4d**/PPO CT-complex (λ max : 412 nm)

resulted in a maximum yield of monomethylaniline and PP.

Thioanisole **4e** also was smoothly photo-oxygenated by PPO to give the corresponding sulfoxide *via* the initial SET process.[8e]

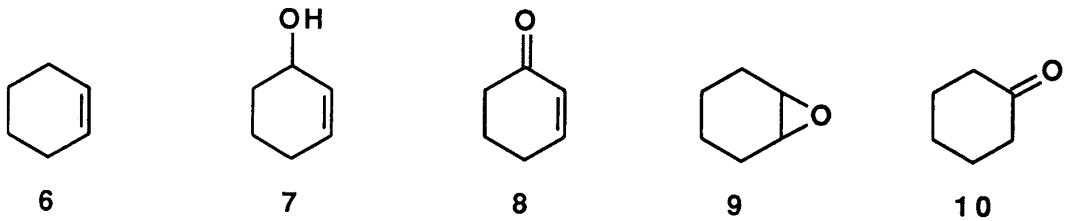
Comparative experiments using MPO and 3,10-dibutylisoalloxazine 5-oxide[14] showed that the photo-oxygenation of phenols by PPO is of a mechanistic nature involving the initial SET *via* an excited CT-complex formation.[8e]

Photo-oxygenation of polycyclic aromatic hydrocarbons (PAHs), such as naphthalene, phenanthrene, pyrene, and benzo[*a*]pyrene, was examined in comparison with that of **4a**.[8f]

These PAHs consumed PPO more smoothly than did **4a** under irradiation to give the corresponding phenols and/or further oxidized products, as expected from the fact that their oxidation potentials are lower than that of **4a**. The photo-oxygenation of the PAHs by PPO was shown to occur predominantly at the most reactive position of the corresponding PAHs cation radicals. These and other experimental results led us to conclude that the PAHs are oxygenated *via* the SET from the PAHs to PPO followed by oxygen atom transfer between the resulting radical ion pairs. At variance with the case of **4a**, however, the SET takes place via an excited CT-complex formed between the PAHs and PPO.

2. Photo-oxygenation of Olefins

Simple olefins such as cyclohexene **6** ($E_{1/2}^{ox}$: 2.14 V *vs* SCE) were oxygenated by PPO under photochemical conditions to give various oxidized products **7-10** (see Scheme 5).[7] Comparative experiments with MPO and physicochemical studies clearly showed that the simple olefins were oxygenated *via* the SET process to singlet-excited PPO, similar to the case of benzene derivatives. In general, any organic molecule with an $E_{1/2}^{ox}$ of 2.2 V or less (vs SCE) can be predicted to be susceptible to oxidation *via* a photo-induced SET process.[15] The photo-oxygenation of **6** by PPO is a case of this type.



6 **7** **8** **9** **10**

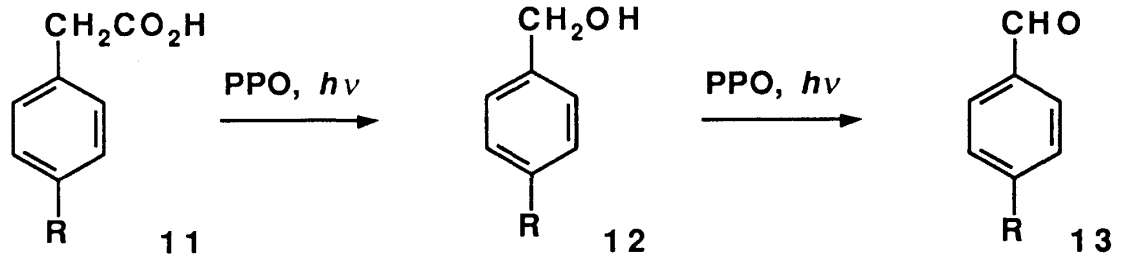
Scheme 5

Analogously, irradiation of styrene in the presence of PPO resulted in the formation of styrene oxide and phenylacetaldehyde.[16]

3. Photo-oxidative Decarboxylation of Arylacetic Acids.

There have been ample precedents for photochemical decarboxylation of arylacetic acids in the

presence of various electron-acceptors involving photo-induced SET followed by decarboxylation.[12a] Thus, in the photoreaction with PPO, the occurrence of a facile oxidative decarboxylation of the arylacetic acids involving the SET process is anticipated.[17]

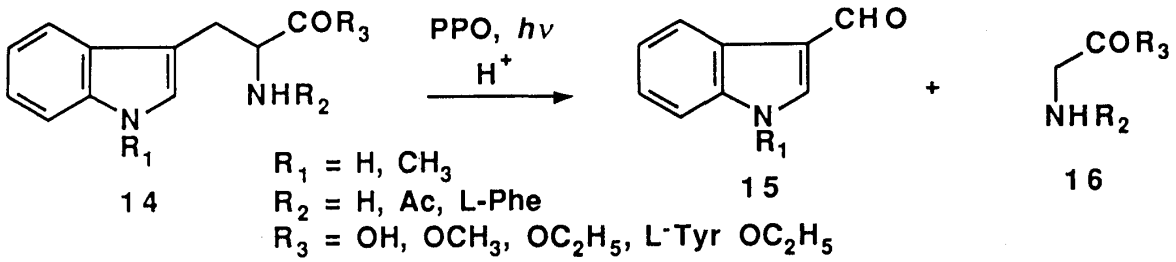


Scheme 6

Irradiation of phenylacetic acids **11** in the presence of PPO gave with ease the corresponding benzaldehydes **13** as the major product *via* an intermediary formation of benzyl alcohols **12**. Both stages of decarboxylative oxygenation and dehydrogenation were proved to involve the SET process from the substrates, **11**, and from the produced benzyl alcohols, **12**, to the singlet-excited PPO.[17a] Analogously, the photo-oxidative decarboxylation of indole-3-acetic acid leading to indole-3-carboxaldehyde proceeded *via* two stages involving the SET process.[17b]

4. Photo-oxidative Cα-Cβ Bond Cleavage of Tryptophan Side-chain

Tryptophan derivatives **14** undergo smoothly the oxidative Cα-Cβ bond cleavage by PPO on irradiation to give 3-indolecarboxaldehydes **15** and the corresponding glycine derivatives **16**, which provides a new method for the modification of tryptophans.[18] The photoreaction is accelerated by the addition of a catalytic amount of acid.



Scheme 7

Although the detailed mechanism, including the role of the acid catalyst, remains uncertain, it is clear that the photoreaction proceeds *via* the SET process in an excited CT-complex between **14** and PPO.

Photochemical Dehydrogenation by PPO

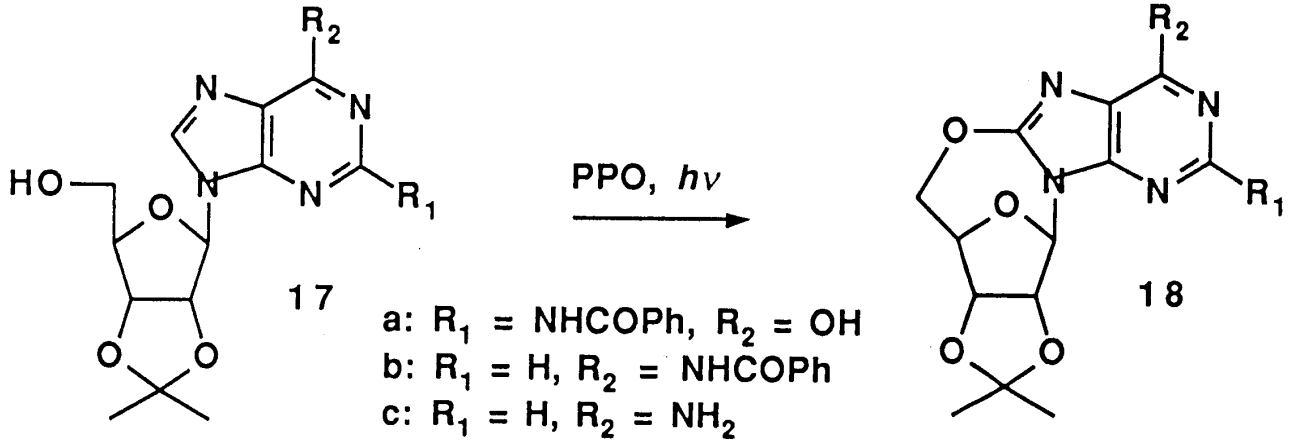
As mentioned in the foregoing section, we observed the concurrent occurrence of photochemical dehydrogenation in the photo-oxygenation of substrates, such as **4b**, **6**, **11**, and **14**, by PPO. For

example, it was shown that the photo-dehydrogenation of benzyl alcohols **12**, cyclohexenol **7**, and indole-3-methanol by PPO proceeds *via* an initial SET process to give the dehydrogenated products.

In this section, further typical examples of the photo-dehydrogenation by PPO are described.

1. Photo-oxidative Cyclization of Purine or Pyrimidine Nucleosides leading to Cyclonucleosides

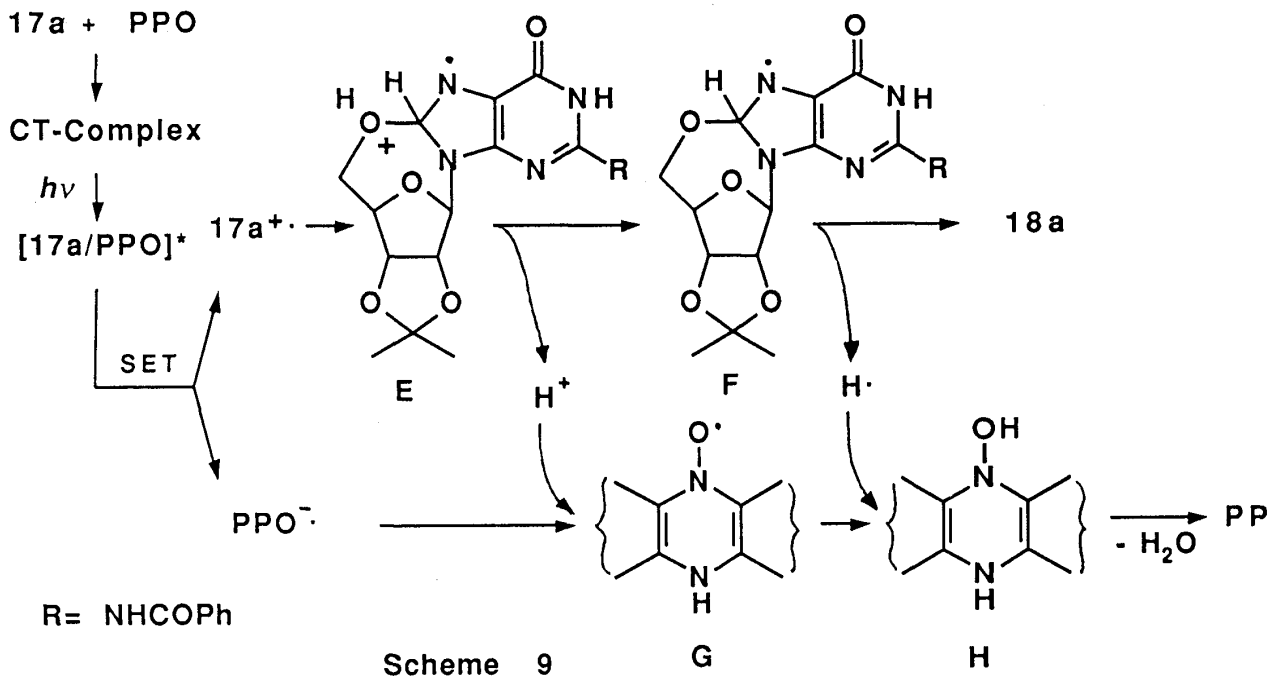
Irradiation of appropriately protected purine nucleosides **17a-c** in the presence of PPO results in the exclusive formation of the corresponding 5′-*O*,8-cyclopurine nucleosides **18a-c**.[19a]



Scheme 8

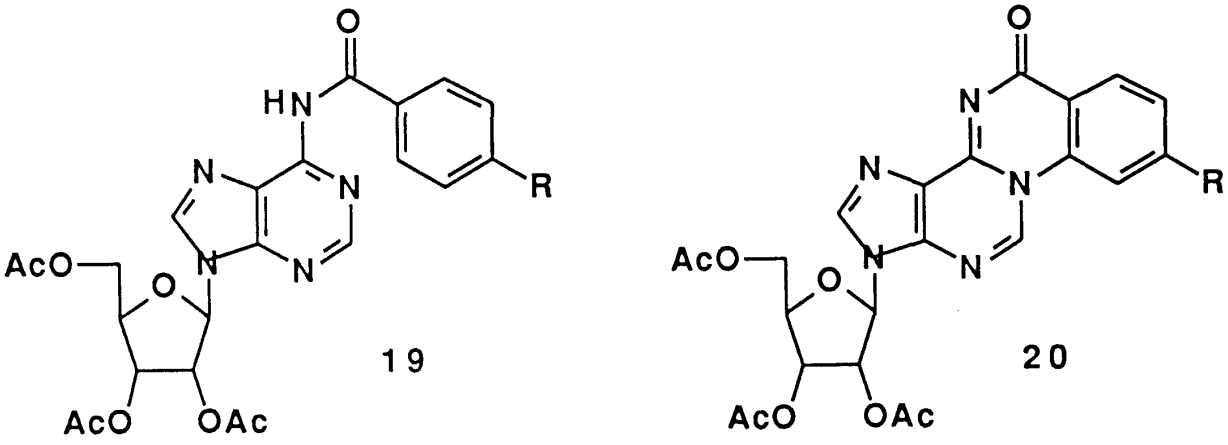
Guanosine derivative **17a** ($E^{ox}p = 1.54$ V *vs* SCE in MeCN) undergoes most efficiently the oxidative cyclization. In adenosine derivatives, prominent N^6-substituent effects on the photo-oxidation by PPO were observed : the consumption of adenosines was accelerated in the order of $N^6Me_2 > N^6HMe > N^6H_2 > N^6HCOPh$, and in the cases of N^6HMe- and N^6Me_2-adenosines, oxidative demethylation occurred as a minor reaction[19b]. Thus, the ease of the reaction correlates well with the electron-donating capacity of the base moiety in the nucleosides employed. In the case of analogously protected uridine ($E_P^{ox} > 2.20$ V *vs* SCE), no formation of cyclonucleoside was observed.

In the photoreaction of **17a** with PPO, irradiation at 388 nm (CT band) resulted in the maximum yield of 5′-*O*,8-cycloguanosines. Thus, a conceivable mechanism for the photochemical 5′-*O*,8-cyclization of **17a** as an example is outlined in Scheme 9, involving the initial SET process followed by trapping of the resulting cation radical (**17a**$^{+\cdot}$) by the 5′-hydroxyl group. Proton transfer of the resulting intermediates **E** to **PPO**$^{-\cdot}$ generates radical **F** and nitroxyl radical **G**, and subsequent hydrogen abstraction from **F** by **G** leads to the formation of **18a** and PP *via* an intermediate **H**.



Scheme 9

2. Photo-oxidative Cyclization of N^6-Benzoyl Adenosines leading to the Quinazolinopurine Ring System

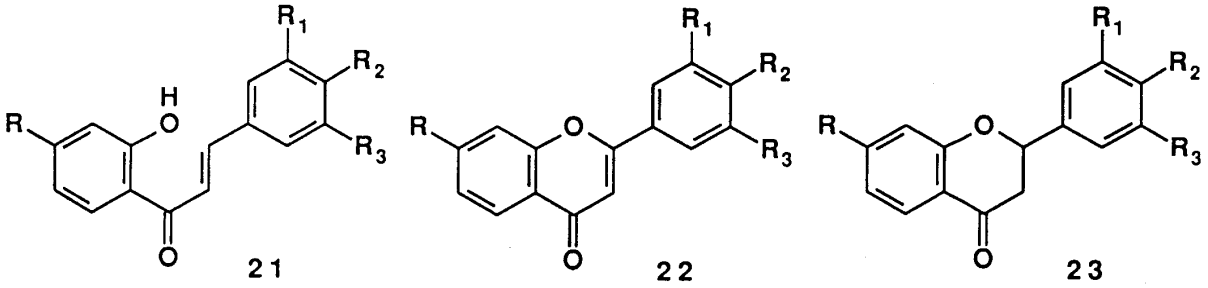


Scheme 10

We found that 2', 3', 5' -tri-*O*-acetyl-N^6-benzoyladenosine derivative **19**, which is protected in the 5' -hydroxyl group, undergo an intriguing oxidative photocyclization to give quinazolino[2,1-i]purine nucleosides **20** by irradiation in the presence of PPO.[20]

In this case, PPO evidently plays a role as an oxidant for the dehydrogenation. In fact, this oxidative cyclization occurs in the presence of other oxidants such as TCNE and *p*-dinitrobenzene.

3. Photo-oxidative Cyclization of 2'-Hydroxychalcones leading to Flavones



R = H, OCH_3 R_2 = H, CH_3, OCH_3, Br, Cl
R_1 = H, OCH_3 R_3 = H, OCH_3

Scheme 11

Irradiation of 2'-hydroxychalcones **21** with UV-visible light in the presence of PPO resulted in the formation of the corresponding flavones **22** and flavanones **23**. Although the photochemical isomerization of **21** leading to flavanones **23** took place concurrently, flavanones **23** was shown not to be an intermediate for the flavone formation. The plots of the consumption-rate constants (-log k) of PPO vs the cathode peak potentials (E_P^{OX}) of the 2'-hydroxychalcones **21** indicated an excellent linear relationship (r= 0.997, ρ = 0.705). This relationship with the positive ρ value is suggestive of the involvement of the SET process in the present photoreactions. The dehydrogenation can be considered to occur in a similar manner to the case of the formation of cyclonucleosides.[21]

Photo-oxygenation of Alkanes by PPO

Photo-oxygenation of alkanes by heterocyclic *N*-oxides such as MPO and pyridine *N*-oxide leading to the corresponding alcohols has provided positive proof[1,10] supporting liberation of an oxygen atom in the triplet-excited state.

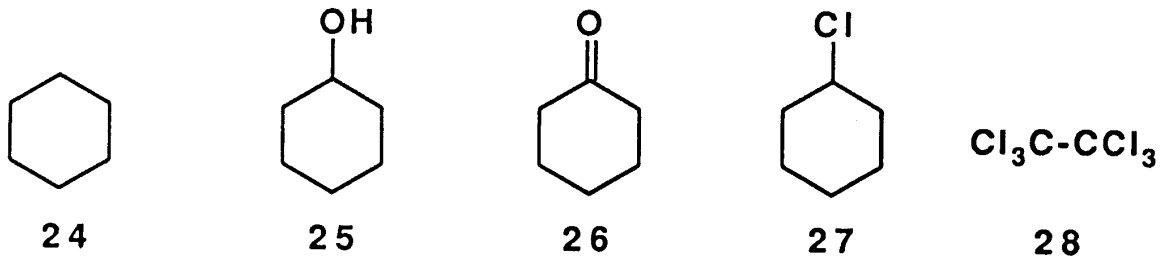
In sharp contrast to MPO, PPO is quite stable in cyclohexane **24** as well as MeCN[8] under UV-irradiation, providing strong chemical evidence in support of no oxene liberation from excited PPO.

However, when chloroform was used as a solvent, PPO oxygenated **24** photo-chemically in a new manner different from the oxene-mechanism.[22]

A mixture of PPO and **24** in dry MeCN, methylene chloride, chloroform, or carbon tetrachloride was irradiated. Only in the case of chloroform, a significant amount of PPO was consumed and formation of cyclohexanol **25**, cyclohexanone **26**, cyclohexylchloride **27**, hexachloroethane **28** together with PP and some undetermined products originating from PPO was observed. The formation of **28** evidently indicates the generation of a trichloromethyl radical during the reaction.

In contrast to the case of PPO, when the mixtures of MPO (UV, λ max : 323 nm) and **24** in the above four solvents were irradiated at approximately 323 nm, MPO was completely consumed and

the oxygenated products, **25** and **26**, were obtained independent of the nature of solvents used. When chloroform was used as a solvent, neither **27** nor **28** were formed.



24 **25** **26** **27** **28**

Scheme 12

Discrepancy in the solvent dependence and the product distribution between the photooxygenations of **24** by both *N*-oxides, PPO and MPO, clearly show that PPO oxygenates **24** *via* a reaction mode entirely different from the case of MPO (the oxene mechanism).

A reaction sequence for the photoreaction of **24** with PPO in chloroform can be considered as follows: the capacity of the excited PPO, probably a triplet-excited state, to abstract a hydrogen atom is sufficient for chloroform, but not for **24**, to generate a trichloromethyl radical and a nitroxyl radical (see **G** in Scheme 9). The former radical readily abstracts a hydrogen atom from **24** to give a cyclohexyl radical which couples with the nitroxyl radical to give **25**.

Unified View of the Photochemistry of PPO in Acetonitrile and Chloroform

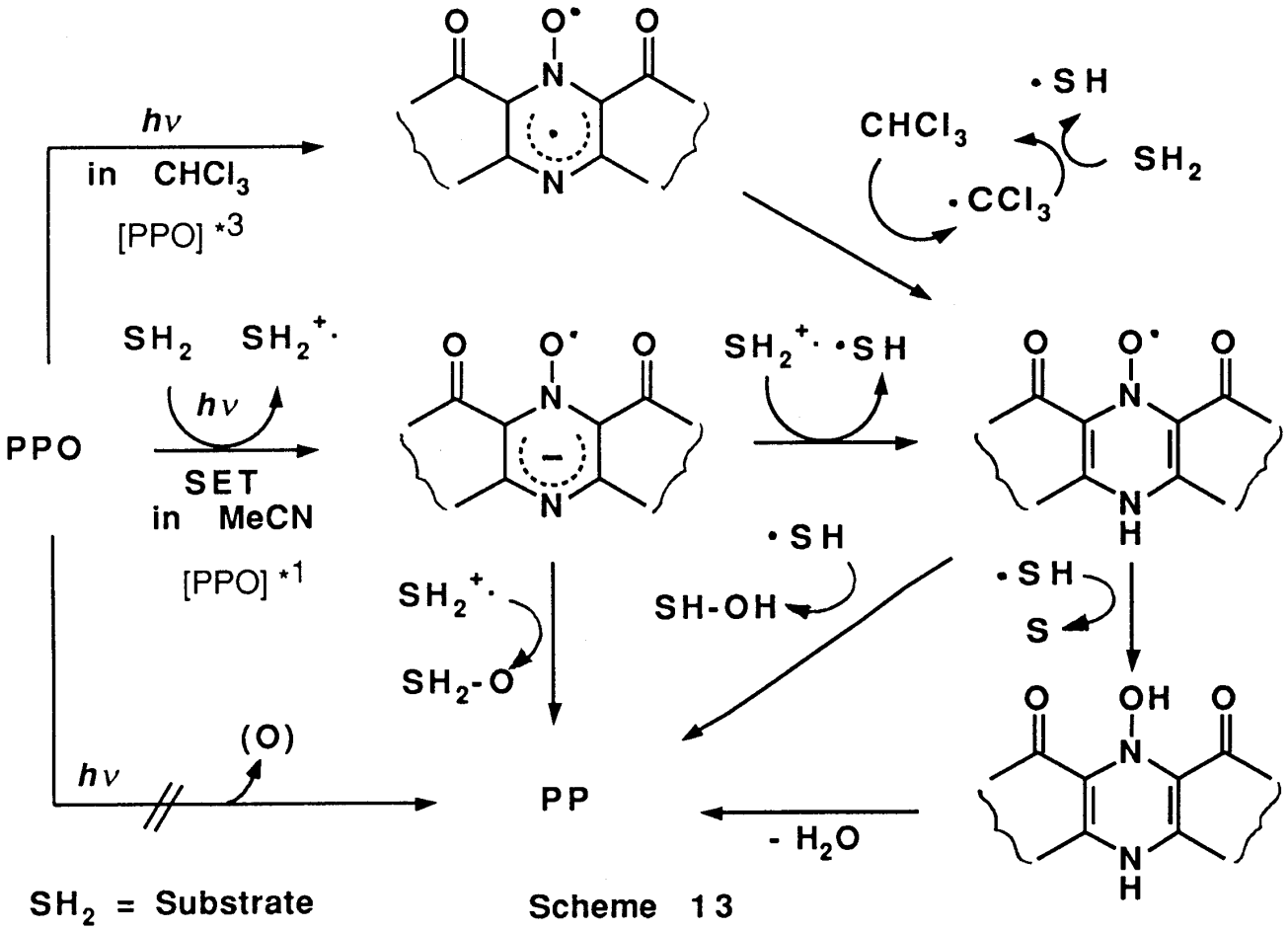
On the basis of the above mentioned results, a unified view of the photochemistry of PPO in acetonitrile and chloroform is summarized as shown in Scheme 13.

Substrates with low oxidation potentials are oxygenated or dehydrogenated *via* the SET process from the respective substrates to the singlet-excited PPO or *via* an excited CT-complex, depending on the nature of the substrates. In contrast to the heterocyclic *N*-oxides so far investigated, PPO acts as an efficient oxidant without accompanying photochemical intramolecular rearrangements and does not liberate an oxygen atom.

In sharp contrast to MPO and pyridine *N*-oxide, PPO is ineffective in MeCN for the photooxygenation of substrates with high oxidation potentials, such as alkanes. When chloroform is employed as a solvent, however, PPO photochemically oxygenates the alkanes *via* the radical chain reaction mediated by chloroform, for which triplet-excited PPO may be responsible.

The Photochemistry of PPO as a Functional Chemical Mimic for the Hemin-Catalyzed Biological Oxidation

Cytochrome *P*-450s are ubiquitous in nature. Because of the diversity of substrates and the variety of transformations that these enzymes execute, this family of cytochromes has attracted much attention from researchers in many fields.



Scheme 13

In the past decade, the chemical mechanism for biological oxidations by cytochrome *P*-450 has been discussed on the basis of various model studies.[2] An active oxygen species is now considered to be a high-valent iron oxenoid symbolized by $Fe^{V}=O$,[2] which possess a multifunctional oxygenating property. Recently, dehydrogenation activity of $Fe^{V}=O$ has been also observed in an activated iron-bleomycin system.[23]

As unified in Scheme 13, PPO also oxidizes various organic substrates under photochemical conditions. Thus, photochemical oxidations by PPO are superior to those of the heterocyclic *N*-oxides investigated thus far from the viewpoint of its efficiency, multi-functionality, and reaction mechanism as a chemical mimic of biological oxidations involving $Fe^{V}=O$ as an active oxygen species.

Metabolic activations of styrene and benzo[*a*]pyrene by cytochrome *P*-450 have been extensively studied in view of their carcinogenicity and mutagenicity.[2b, 24, 25]

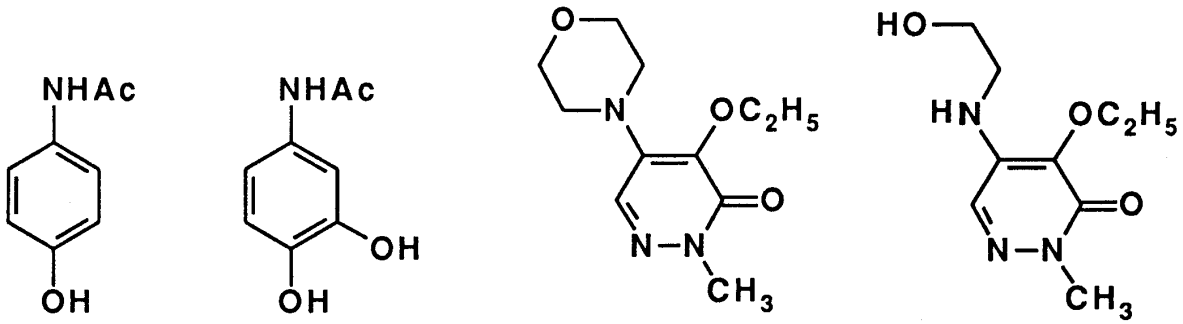
Photoreactions of styrene[16] with PPO resulted in the formation of styrene oxide and phenylacetaldehyde, which is in agreement with biological oxidation products. In the case of benzo[*a*]pyrene,[8f] photo-oxidation by PPO gave a mixture of 1,6-, 3,6-, and 6,12-benzo[*a*]pyrenediones *via* an initial oxygenated product, 6-hydroxybenzo[*a*]pyrene. This can be regarded as a simple reaction mimic for one of the metabolic activation processes involved in carcinogenesis of

benzo[*a*]pyrene.

As described in previous sections, the photo-oxidative decarboxylation of indole-3-acetic acid,[17b] a plant growth hormone, the photo-oxidative Cα-Cβ bond cleavage of the tryptophan side-chain,[18] and the photo-oxidative cyclization of chalcone **21**[21] mimic well the bio-metabolism of these substrates.[26]

Cyclohexene **6** and norbornene were photochemically oxygenated by PPO to give products similar to the case of the hemin-catalyzed oxidations,[27] which are a model reaction of cytochrome *P*-450.

The photo-oxidation of drugs by PPO may be applicable as a complementary tool for drug metabolism studies. For example, the photo-oxygenation of acetaminophen **29**, a widely used analgesic and antipyretic drug, by PPO[8c] gave 3-hydroxyacetaminophen **30**, which is one of the metabolites. The photo-oxidation of Emorfazone **31**, an analgesic antiinflammatory drug,[28] by PPO gave the oxidative ring-opening product **32** as a major product.[29] The photo-product **32** is identical with a major metabolite of **31** in animals. These results show that photo-oxidation by PPO is a useful and convenient tool in drug metabolism studies.



29 **30** **31** **32**

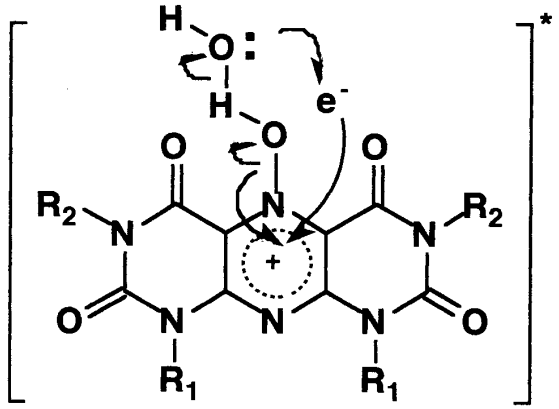
Scheme 14

Facile Bimolecular Generation of OH Radicals by Photolysis of PPOM in Water

While a variety of methods for the generation of OH radicals are precedent,[30] the practical use of photo-excited heterocyclic *N*-oxides as an OH radical source has not been fully documented.

The reason for this is that heterocyclic *N*-oxides generally undergo intramolecular rearrangements rather than oxidations of co-substrates under photochemical conditions. As mentioned in previous sections, PPO functions in almost all cases as an electron acceptor in the initial stage of the photoreactions and subsequently acts as a high efficiency multi-functional oxidant. These results suggest that a water soluble derivative of PPO, PPOM, may efficiently generate OH radicals in a bimolecular manner from its protonated excited state in water, as illustrated in Scheme 15.

When an aqueous solution of PPOM was irradiated with UV-visible light (>355 nm), PPOM was smoothly consumed to give a quantitatively deoxygenated product, PPM. Although addition



Scheme 15. Proposed Mechanism for the Bimolecular Generation of Hydroxyl Radicals by Photolysis of Pyrimido[5,4-*g*]pteridinetetrone *N*-Oxides in Water

of dimethyl sulfoxide (DMSO) did not affect significantly the consumption of PPOM, the colorimetric quantitative assay[31] which involves trapping of the produced methane sulfinic acid by Fast Blue BB showed the generation of approximately two equimolar amounts of OH radicals. The generation of OH radicals was also evidenced by ESR spin-trapping using DMPO.

Since the ^{18}O-labeled PPOM, PP^{18}OM, was prepared with ease, ^{18}O-labeled OH radicals can be readily generated upon irradiation of PP^{18}OM in water.

The formation of 8-oxopurine nucleosides by the reactions of purine nucleosides with OH radicals is well documented.[32] In fact, photolysis of PPOM in the presence of adenosine in water gives 8-oxoadenosine and undetermined products.[5]

Above results clearly indicate that PPOM serves as a clean and quantifiable OH radical generator which operates on irradiation with long-wavelength light (>355 nm) in water without any additive.[6]

Thus, we investigated one of whose utilizations as an photochemical DNA cleaver. (see Table 1 and its footnotes) Table 1 shows that nicking of Form I DNA occurs in a concentration dependent manner with respect to PPOM and is inhibited by addition of an OH radical scavenger, such as DMSO. Essentially complete conversion of Form I DNA to Form II DNA was achieved at 2.0 μM concentration of PPOM after irradiation in a buffer solution for 10 min. Comparative experiments using phenazine *N*-oxide and alloxazine *N*-oxide showed the superior efficiency of PPOM as a photo-chemical DNA cleaver.[5] Recent work[30a] has demonstrated unimolecular generation of OH radical from phthalimide hydroperoxide with UV light and its use as a photochemical DNA cleaver. Our *N*-oxide PPOM is more efficient and convenient for use as an agent for OH radical generation and DNA cleavage.

Table 1. Cleavage of Supercoiled Circular ΦX 174 RF I (Form I) DNA into Nicked Circular (Form II) DNA by Photoirradiation of PPOM and its Inhibition with a OH Radical Scavenger, i.e., Dimethyl Sulfoxide (DMSO)[a]

PPOM	Content of DMSO	% Form I DNA[b]	% Form II DNA[b]
0.5μM	-	43	54
1.0μM	-	5	92
	0.1%	86	13
	1.0%	92	7
2.0μM	-	ND	98
	0.1%	47	52
	1.0%	70	29

a. The reaction mixtures (30μl total volume) containing 200 ng form I DNA and PPOM at varying concentrations in 50 mM sodium cacodylate buffer (pH 7.5) were irradiated in the absence or presence of DMSO at a distance of 5 cm from 400W high-pressure mercury arc lamp through a $BiCl_3$ solution filter (>355 nm) at ambient temperature for 10 min and then analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The employed DNA contains a small amount of form II DNA (~10%) and a trace amount of linear DNA.

b. Yields were estimated by densitometric analysis of a negative photograph of the agarose gel after ethidium bromide staining.

Concluding Remarks and Future Opportunities

The extraordinary photochemical properties of PPO and PPOM among the heterocyclic *N*-oxides evidently arise from their special structural feature and made it possible for them to function as an agent for oxygenation or dehydrogenation and as an OH radical generator. The multi-oxidative functions of the *N*-oxides under photochemical conditions seems to mimic formally those of iron-oxenoid and oxygen species.

The further structural manipulation of PPO and PPOM is now in progress because photoreactions using this class of *N*-oxides is promising for use as tools for chemical modification of proteins and nucleic acids based on the results described in this account. In particular, hydrophilic PPO derivatives such as PPOM will contribute to design of a new class of DNA cleavers as well as to studies on the reactions of OH radicals with biological substances.

Thus, the scope of application of such *N*-oxides will become broader in the fields of organic, bioorganic, and medicinal chemistry.

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