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NEW SINGLET OXYGEN SOURCE AND TRAPPING REAGENT FOR PEROXIDE INTERMEDIATES

I. SAITO,† R. NAGATA, H. NAKAGAWA, H. MORIYAMA, T. MATSUURA and K. INOUE‡

Department of Synthetic Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606, Japan,‡ Department of Industrial Chemistry, Faculty of Engineering, Ehime University, Matsuyama 790, Japan

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Application of newly-developed water-soluble singlet oxygen sources in the oxidation of biologically important compounds and the electron-transfer process involving singlet oxygen has been reviewed. Particularly, oxidation products of tryptophan by chemically generated singlet oxygen were compared to those obtained in dye-sensitizd photooxygenation. The usefulness of trimethylsilyl cyanide as a trapping reagent for dipolar peroxide intermediates has been demonstrated in the photooxygenation of N-methylindoles, 2-(methoxymethylene)adamantane and adamantylideneadamantane in aprotic solvents. Based on these trapping reactions mechanism of singlet oxygen reaction of electron-rich enol ethers and enamines is discussed in light of theoretical calculation.

KEY WORDS: Chemical singlet oxygen source; superoxide generation; zwitterionic peroxide; tryptophan; trimethylsilyl cyanide.

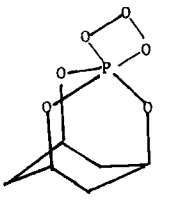
INTRODUCTION

A convenient method for generating pure singlet oxygen under mild conditions in aqueous system is highly desirable for the mechanistic study of singlet oxygen ($^1O_2$) reaction of biological substrates. Although a number of methods have been known for generating $^1O_2$,[1] most of these methods are not ideally suited for kinetic studies of $^1O_2$ reactions in aqueous solution. For example, dye-sensitized photochemical method must be used only with special precaution, since dye may also sensitize substrate oxidations that are not $^1O_2$ mediated.[2] Chemical $^1O_2$ sources that give no other oxidizing species beside $^1O_2$ are more desirable for $^1O_2$ reaction of biological substrates, particularly for their mechanistic investigations. In the present paper we describe the use of newly-developed water-soluble $^1O_2$ sources in the oxidation of biologically important substrates in aqueous solution.[3] In addition, by using water-soluble $^1O_2$ sources generation of $O_2^{\cdot-}$ from $^1O_2$ by single electron-transfer is demonstrated.[4] We also describe the utility of trimethylsilyl cyanide as a specific reagent for trapping dipolar peroxide intermediates formed in $^1O_2$ reaction of electron-rich double bond systems in aprotic solvents.[5]

† To whom correspondence should be sent.

RESULTS AND DISCUSSION

Water-soluble ozonide, 1-phospha-2,8,9-trioxaadamantane ozonide (**1**), was first demonstrated by Schaap *et al.*,[6] as a chemical $^1O_2$ source. Owing to the thermal instability ($k_{decomp} = 1.06 \times 10^{-3}\,sec^{-1}$ at $-17°C$),[6] the water soluble ozonide is not ideally suited for $^1O_2$ source in aqueous solution. Several years ago we demonstrated the usefulness of water-soluble naphthalane endoperoxide **2** as a chemical $^1O_2$ source.[3] This compound is soluble in phosphate buffer (pH 7.5) up to 10 mM and thermodissociates to $^1O_2$ ($^1O_2$ yield > 82%) and parent naphthalene with a half-life of 23 min at 35°C.[3,4] We also devised a new polymer $^1O_2$ carrier **3** that can bind and release $^1O_2$ reversibly near room temperature for the use as a synthetic reagent as well as for the potential application to insect or bacteria-repellent systems, owing to the high toxicity of $^1O_2$ toward cells.[7] For the purpose of oxidation of guanine residues of polynucleotides, naphthalene endoperoxide linked to acridine such as **4** was prepared.[8] Very recently Nieuwint *et al.*,[9] have reported the use of endoperoxide of disodium 3,3′-(1,4-naphthylidene)dipropionate (**5**) for the oxidation of covalently closed circular DNA in aqueous solution. By a different approach, Midden and Wang[10] have demonstrated a clean and simple method for generating $^1O_2$ using photosensitization in which a suitable sensitizer is immobilized on the surface of a glass plate. This clean method for photochemically generating $^1O_2$ has been used for the kinetic studies of the reactions of complicated biological systems. However, this technique cannot be used for photolabile systems.



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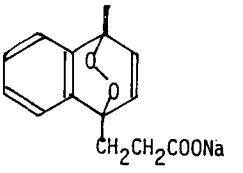

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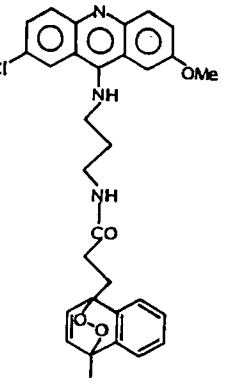

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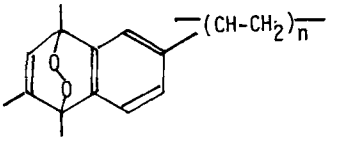

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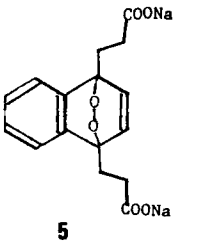

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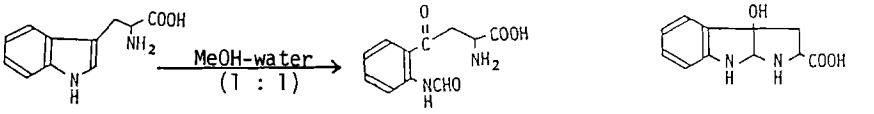
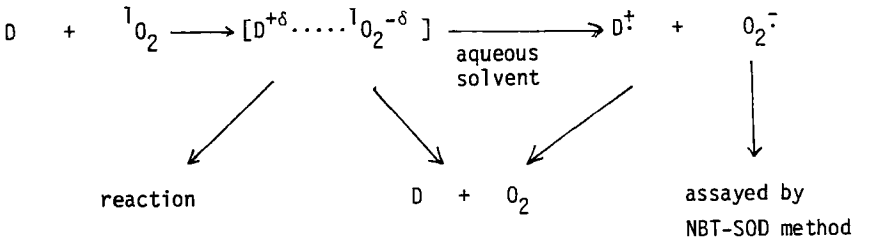
The usefulness of the water-soluble $^1O_2$ source **2** in the oxidation of biological substrates is typically illustrated by the oxidation of tryptophan (Trp). The dye-sensitized photooxygenation of Trp is known to provide N-formylkynurenine (**6**) and 3a-hydroxypyrrolidinoindole (**7**) via 3a-hydroperoxypyrrolidinoindole.[11,12] Previous kinetic study has revealed that the dye-sensitized photooxygenation of Trp is highly dependent on the type of sensitizer used.[13] The photooxygenation of Trp with rose bengal (RB) proceeds exclusively via $^1O_2$ mechanism, whereas with thionine it proceeds by a combination of $^1O_2$ and Type I mechanisms.[13] When Trp (0.4 mM) was oxidized in aqueous methanol at 35°C in the presence of **2** (2.8 mM), **6** and **7** were obtained in 23 and 42% yields, respectively.[3,13] The product ratio is essentially the same as that obtained in RB-sensitized photooxygenation using a filter solution (aqueous $CaCl_2$-$CuCl_2$ solution, cutoff < 550 nm). In contrast, thionine-sensitized photooxygenation gave a different product ratio.[13] The low yield of **7** in thionine-sensitization may be ascribed to the co-occurrence of a free radical process (Type I mechanism). In both cases irradiation without filter solution results in a diminished yield of **7** due to the further photodecomposition of **7** by shorter-wavelength.[13]



Trp	**6**	**7**
2 (7 equiv)	23%	42%
RB/O_2/ > 550 nm	23	44
RB/O_2/ > 300 nm	30	1
thionine/O_2/ > 550 nm	22	23
thionine/O_2/ > 300 nm	20	–

By using water-soluble $^1O_2$ source **2**, we devised a convenient method for detecting the $O_2^{\cdot}$ formation from $^1O_2$ reactions of readily oxidizable substrates in aqueous solution.[4,14] The method employs **2** and a superoxide-detecting reagent such as a combination of *p*-nitrotetrazolium blue (NBT) and superoxide dismutase (SOD). The generation of $O_2^{\cdot}$ in the reactions of a variety of electron-rich substrates (*D*) such as substituted N,N-dimethylanilines,[4] thioanisole,[15] hydrogenated nicotinamide adenine dinucleotide (NADPH)[14] and 5-hydroxytryptophan[14] was confirmed by this method. These substrates have oxidation potentials ($E^{ox}_{1/2}$) less than 0.5 V (SCE). Substrates with $E^{ox}_{1/2}$ larger than 0.5 V (SCE) did not produce $O_2^{\cdot}$ even under polar aqueous conditions.[14] Thus the oxidation potential ($E^{ox}_{1/2} = 0.5$ V) seems to be the borderline for the generation of $O_2^{\cdot}$ from $^1O_2$ via single electron-transfer in aqueous system. There are many electron-rich substrates with oxidation potentials less than 0.5 V in biological systems such as mitochondrial components.[16] Such an electron-transfer to photochemically generated $^1O_2$ from electron-donors has been confirmed by Rodgers[17] and Foote[18] by laser flash technique. A combination of water-soluble chemical $^1O_2$ souces and $O_2^{\cdot}$-detecting reagents is more routinely usable as a mechanistically less complicated method for detecting $O_2^{\cdot}$ in $^1O_2$ reaction of complicated biological systems.

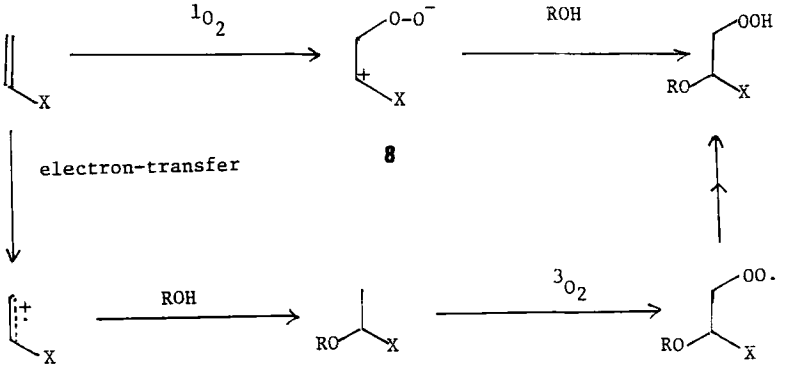


D: electron-donating substrates with $E^{ox}_{1/2}$ less than 0.5 V (SCE)

We also prepared polymer endoperoxides such as **3** by singlet oxygen reaction of poly(vinylnaphthalene) for the use as a polymer-bound chemical $^1O_2$ source.[7] Advantages of polymer $^1O_2$ sources are following: (i) $^1O_2$ is generated at ambient temperature under a variety of conditions such as in a solution, suspension, polymer film and solid; (ii) the polymer is easily removable and reusable. Polymer-bound $^1O_2$ source **3** is particularly useful for oxidation in non-solvent systems.

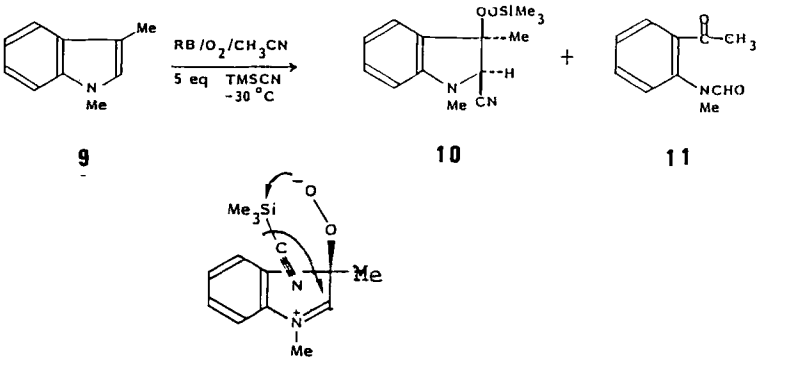
Interception of zwitterionic peroxide intermediates with trimethylsilyl cyanide

Transient 1,4-zwitterionic peroxide intermediates **8** have been proposed in $^1O_2$ reaction of a variety of electron-rich substrates such as enol ethers and enamines.[19] Experimental support for such zwitterionic peroxides thus far obtained is based mainly on the chemical trapping reaction with nucleophilic solvents such as alcohols.[19,20] However, certain electron-transfer-initiated photooxygenation, *e.g.*, dicyanoanthracene (DCA)-sensitized photooxidation, is also capable of producing similar trapping products by interception of substrate radical cations with alcohols and oxygen.[21,22] Thus the trapping with alcohols cannot always be used as an evidence for zwitterionic peroxide intermediates. A more reliable trapping agent, usable in a variety of aprotic solvents, is clearly desirable. We demonstrate that trimethylsilyl cyanide (TMSCN) can serve as a trapping agent for such zwitterionic peroxide intermediates in $^1O_2$ reaction of electron-rich olefins in aprotic solvents.[5]



We found that RB-sensitized photooxygenation of 1,3-dimethylindole **9** in the presence of TMSCN (5 equiv) in dry acetonitrile at −30°C produced the trapping product **10** (70%) together with dioxetane-derived product **11** (17%).[5] Similar trap-

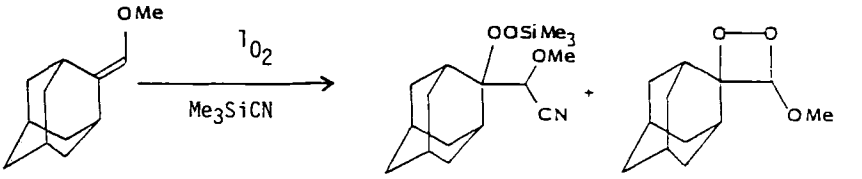
ping products were obtained in tetraphenylporphine (TPP)-sensitized photooxygenation of 1,2-dimethyl-3-isopropylindole and 1,3-dimethyl-2-phenylindole.[5] The exclusive formation of cis adduct may be rationalized by assuming addition of TMSCN to a precursive dipolar peroxide intermediate such as 1,4-zwitterion or perepoxide. The nucleophilic attack by peroxy anion on the silicon would give a pentacoordinated silicon which deposits the cyanide ion at the cationic site of the trapped dipolar species as illustrated as **12**.



9 **10** **11**

12

TPP-sensitized photooxygenation of 2-(methoxymethylene)adamantane (**13**) in the presence of TMSCN (5 equiv) in dichloromethane at $-70°C$ gave the adduct **14** (35%), dioxetane **15** (30%) and 2-adamantanone (22%).[5] RB-sensitized photooxygenation in more polar acetone under the same conditions gave a higher yield (57%) of the trapping product **14**. Control experiments indicated that dioxetane **15** upon reaction with TMSCN never produce **14** under the irradiation conditions but decomposed to 2-adamantanone exclusively. The result provides strong evidence for the existence of a precursive intermediate for **15**, most likely the zwitterionic peroxide. It was reported that the precursor of **15** in the photooxygenation of **13** is captured by acetoaldehyde[23] and methanol[24] as solvent at $-78°C$. In contrast, DCA-sensitized photooxidation of **13** in the presence of TMSCN (5 equiv) in acetonitrile at $-20°C$ never produced the trapping product **14** but gave only 2-adamantanone.



13 **14** **15**

+ 2-adamantanone

In order to know the generality of the trapping reaction, we examined the photooxygenation of several other electron-rich double bond systems. Singlet oxygenation of 2-methyl-2-butene was not affected by the presence of TMSCN. Photooxy-

genation of symmetrical enol ethers such as 1,4-dioxene (**16**), 2,3-diphenyl-1,4-dioxene (**17**) and benzodioxene (**18**) in the presence of a large excess of TMSCN produced none of the trapping products but gave only the dioxetanes and their ring cleavage products. These results suggest that only highly unsymmetrical enol ethers or enamines such as **9** or **13** are interceptable by TMSCN. It is interesting here to compare these results with theoretical prediction. Recent MO calculations[25,26] (*ab initio* and RHF CI (3) methods) have suggested that the concerted [$2_s + 2_a$] mechanism is the most favorable for the addition of $^1O_2$ to symmetrical enol ether and enamine, whereas 1,4-zwitterion is the most stable intermediate in the $^1O_2$ reaction of unsymmetrical enol ether and enamine in polar solvents. The results of trapping with TMSCN are not inconsistent with this theoretical result (Figure 1).

The precursor of dioxetane **20** in photooxygenation of adamantylideneadamantane (**19**) has been reported to be captured by methyl phenyl sulfoxide to give epoxide **22** and sulfone.[27] Perepoxide intermediate **21** has been proposed as the intermediate.[27] We have examined the $^1O_2$ reaction of **19** in the presence of TMSCN. It was reported that TPP-sensitized photooxygenation of **19** gives stable dioxetane **20** exclusively.[28] In the presence of TMSCN TPP-sensitized photooxygenation gave a considerable amount **22** together with dioxetane **20**. As shown in Table I the yield of **22** increased with increasing concentration of TMSCN. On the contrary to the case of **13**, less polar solvent such as benzene and higher temperature seem to favor the formation of epoxide **22**. Control experiments indicated that dioxetane **20** did not react with TMSCN under the photooxygenation conditions and was recovered quantitatively. The mechanism of the formation of epoxide **22** in the presence of TMSCN is not clear

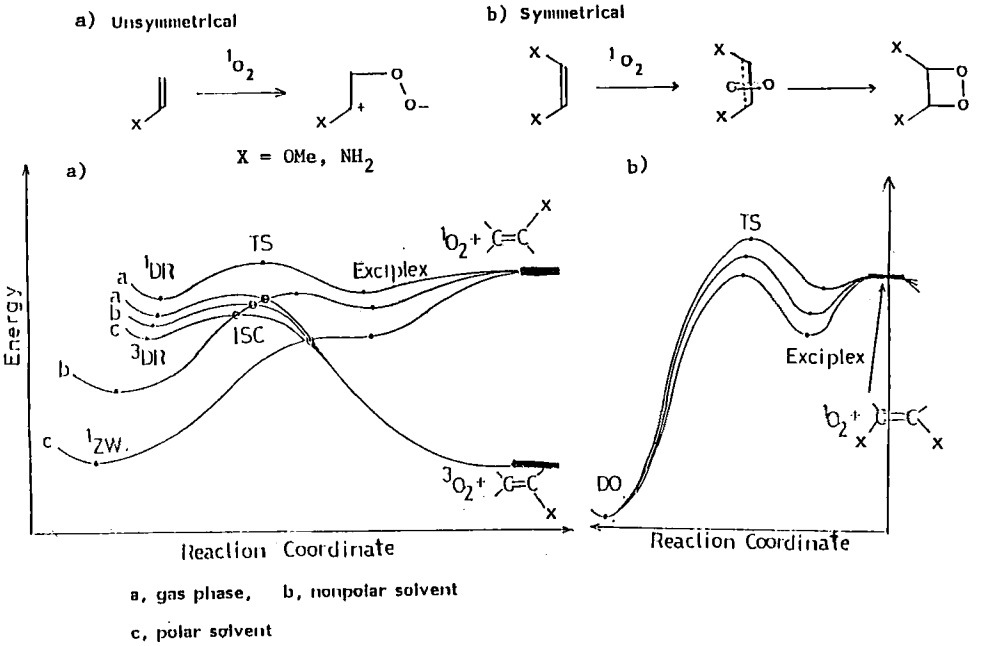


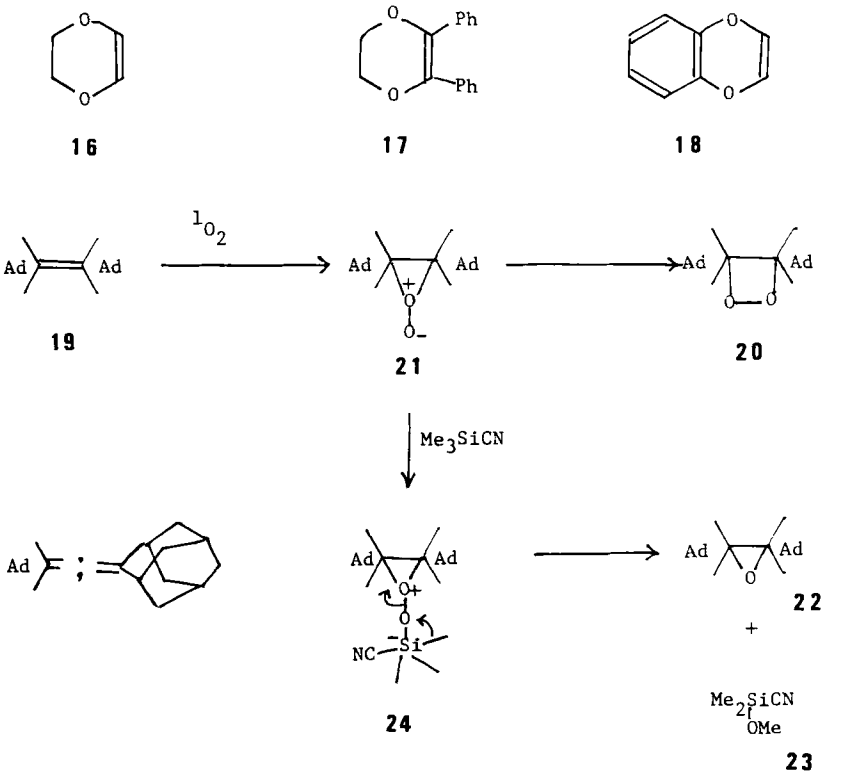
FIGURE 1 Calculated potential energy surface for the addition of singlet oxygen to enol ether or enamine.[25,26]

TABLE I
Tetraphenylporphine-sensitized photooxygenation of adamantylideneadamantane (**19**) in the presence of trimethylsilyl cyanide (TMSCN)[a]

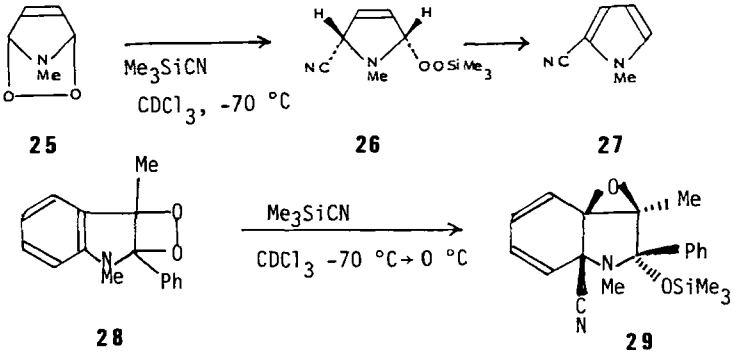
Entry	Solvent	Temperature (°C)	TMSCN (equiv)	Product, yield %[b] **20**	**22**
1	CH_2Cl_2	0	1	82	16
2	CH_2Cl_2	0	3	53	40
3	CH_2Cl_2	0	5	43	55
4	CH_2Cl_2	−10	3	75	26
5	CH_2Cl_2	10	3	50	43
6	benzene	10	3	11	80
7	benzene	10	–	75	–

[a]Each solution contains **19** (4 mM), TPP (0.5 mg) and varying amounts of TMSCN in 5 mL. Irradiation was performed with a 650 W tungsten-halogen lamp through a filter solution (cutoff < 350 nm).
[b]Determined by GLC using *n*-eicosane as standard.

at present. However, it seems possible that nucleophilic attack by anionic oxygen of perepoxide **21** on the silicon would give similarly a pentacoordinated silicon such as **24** which then undergoes 1,2-methyl group shift to give **22** and **23**. However, attempts to detect **23** by GC mass have been fruitless because of the thermal lability of **23** under the conditions.



Finally, it is worthwhile to note here that certain dioxetanes and endoperoxides can react with TMSCN thermally. While dioxetanes **15** and **20** or furan endoperoxide[29] did not react with TMSCN, endoperoxide **25** formed in low-temperature photooxygenation of N-methylpyrrole rapidly reacted with TMSCN to give **26** which on warming to room temperature decomposed to 2-cyanopyrrole (**27**) almost quantitatively.[5] Likewise, unstable dioxetane **28** formed at low-temperature (−70°C) photooxygenation of 1,3-dimethyl-2-phenylindole reacted with TMSCN in dichloromethane at 0°C to give **29** whose structure was established by X-ray analysis. The detail of the reaction will be published in a forthcoming paper.



In summary, the present work demonstrates the utility of trimethylsilyl cyanide as a trapping agent for dipolar peroxide intermediates formed in singlet oxygen reaction of electron-rich substrates in aprotic solvents.

MATERIALS AND METHODS

Materials

L-Tryptophan, rose bengal, tetraphenylporphine and dicyanoanthracene were purchased from Wako Chemicals and are used without further purification. Commercially available trimethylsilyl cyanide was purified by careful fractional distillation. Water-soluble naphthalene endoperoxide **2** was prepared as described previously.[4] 2-(Methoxymethylene)-adamantane (**13**) was prepared by the published procedure.[30] Adamantylideneadamantane (**19**) was prepared from 2-adamantanone by the procedure of McMurry.[31] N-formylkynurenine (**6**) and 3a-hydroxypyrrolidinoindole (**7**) were prepared by the known method.[12] Dichloromethane, acetonitrile and acetone were distilled from CaH_2. Benzene was dried over sodium metal.

^{1}H-N.M.R. spectra were recorded on a Varian T-60 or JEOL GNM-GX 400. GLC analyses were performed on a Shimadzu GC-8A using a 6 ft column packed with Silicon DC 550. HPLC was performed on a Waters ALC/GPC 240 equipped with a radial pack A column.

Oxidation of tryptophan

Oxidation products of tryptophan (Trp) in dye-sensitized photooxygenation in aqueous methanol were described previously.[13] Oxidation of Trp with water-soluble

naphthalene endoperoxide **2** was carried out as follows. A solution of Trp (0.4 mM) and **2** (2.8 mM) in 50% aqueous methanol was purged with nitrogen and sealed. The solution was shaken in the dark at 25°C for 2 h. The reaction mixture was analyzed by HPLC by comparing authentic samples of **6** and **7** prepared independently.

Photosensitized oxygenation in the presence of trimethylsilyl cyanide

The results of RB-sensitized photooxygenation of 1,3-dimethylindole (**9**) was reported previously.[5] The structure of **10** was confirmed by spectroscopic data including ^{1}H-N.M.R. NOE difference technique and by chemical transformation as described previously.[5]

Photooxygenation of 2-(methoxymethylene)adamantane (**13**) in the presence of TMSCN was already reported.[5] The product distribution was assayed by ^{1}H-N.M.R. (**14** and **15**) and GLC (2-adamantanone). ^{1}H-N.M.R. data of **15** and **14** are as follows; **15**: ^{1}H-N.M.R. ($CDCl_3$) δ 1.4–2.10 (*m*, 14 H), 3.43 (*s*, 3 H), 5.45 (*s*, 1 H); **14**: ^{1}H-N.M.R. ($CDCl_3$) δ 0.23 (*s*, 9 H), 1.60–2.50 (*m*, 14 H), 3.52 (*s*, 3 H), 4.60 (*s*, 1 H).

TPP-sensitized photooxygenation of adamantylideneadamantane (**19**) was carried out under the conditions listed in Table I. Dioxetane **20**[28] and epoxide **22**[27] were prepared independently by the published procedures. The product ratio was determined by GLC using *n*-eicosane as standard. GC mass analysis of the reaction mixture was performed by using a JEOL JMS-DX 300 spectrometer.

Acknowledgement

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