REACTIONS OF 9-PHOSPHORYLACRIDINES WITH AQUEOUS HYDROGEN PEROXIDE ACCOMPANIED BY WEAK CHEMILUMINESCENCE: REMARKABLE SUBSTITUENT EFFECT ON EMISSION EFFICIENCY

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Abstract – The reaction of 9-phosphorylacridines with aqueous hydrogen peroxide provides weak chemiluminescence, whose emission efficiency depends on the structures of the phosphorus groups. The bulky substituents in the phosphorus groups increased the chemiluminescence quantum yields, which can be explained by assuming that the present chemiluminescence reaction involves a CIEEL process *via* phospha-1,2-dioxetanes as the high-energy intermediates.

Much attention is being denoted to chemiluminescence because of its practical usefulness as well as scientific interest.[2] In a variety of chemiluminescence reactions, *N*-methylacridone plays the role as an emitter because of its strong fluorescence. For example, electronically excited *N*-methylacridone generated chemically from the reactions of acridinium salts [3] or lucigenine [4] with hydrogen peroxide, emits blue light. These chemiluminescent compounds are being used as immunoassay reagents. [5] On the other hand, White *et al*. [6] reported that the reaction of phenyl 9-acridinecarboxylate (**1**) with aqueous

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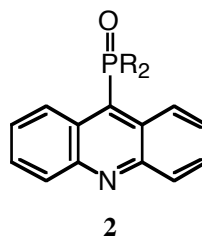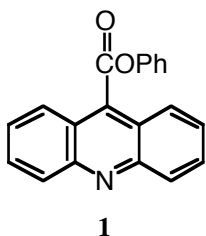
hydrogen peroxide also displayed chemiluminescence in which the emitter was either acridone or its anion depending on the reaction conditions. In connection with the chemiluminescence of these acridine derivatives, we are interested in the reaction of 9-phosphorylacridines (**2**) and wish to know whether these acridines bearing the phosphorus groups are chemiluminescent, and if so, how the chemiluminescence reaction involving the phosphorus chemistry proceeds. In this study, we investigated the chemiluminescence reactions of **2** and found a remarkable substituent effect on the light emission efficiency, which was helpful when discussing the reaction mechanism.

The Arbuzov reaction of acridinium hydrobromide and the corresponding phosphites gave *N*-hydro-9-phosphorylacridanes, [7a] which were oxidized with chloranil to yield 9-phosphorylacridines (**2a-e**) [7a,b,c] as shown in Scheme 1. The reaction of these acridines with alkaline hydrogen peroxide in aqueous THF or DMSO at a high concentration provided a very weak chemiluminescence observable with the naked eye in the dark. No light emission was detectable under neutral conditions. The absorption spectrum of the resulting solution (aqueous DMSO) exhibited three peaks at 396, 416 and 460 nm, which was not for the neutral acridone but for its anion. The acridone anion absorbs in the longer wave region than its neutral form having two peaks at 380 and 400 nm. The yields of the acridone anion estimated by the absorption spectra of the resulting solution were generally high in these chemiluminescence reactions except for **2d** (vide infra). Detection of only acridone as a strongly fluorescent substance during the reactions supports the fact that the light emission would be ascribed to either an excited acridone anion or neutral acridone, but it was difficult to know which was the emitter since the emission was too weak and too short-lived to be spectroscopically detected.

2a, R=OMe
2b, R=OEt
2c, R=OPr-*i*
**2d**, R=OPh
2e, R=Ph
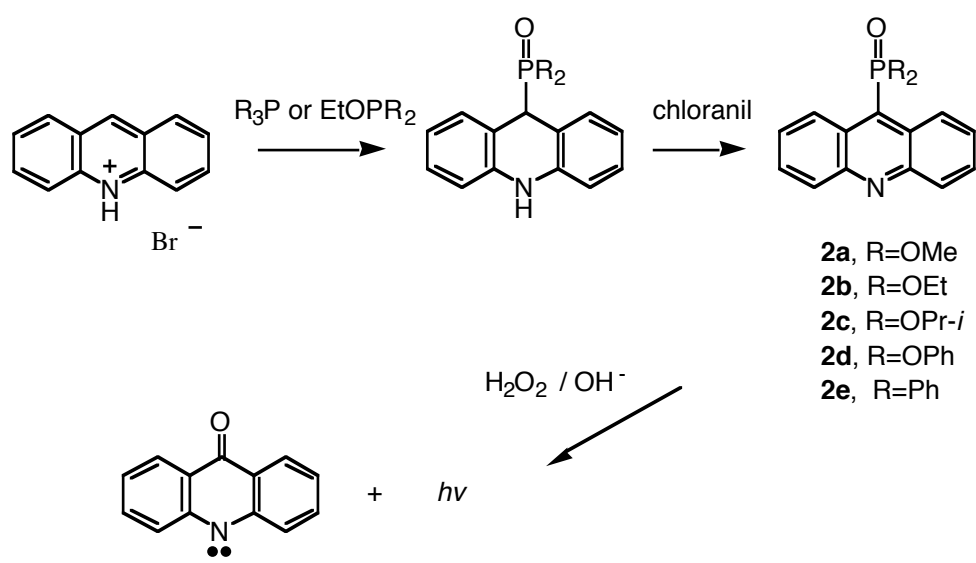
Scheme 1

The chemiluminescence quantum yields in the reactions of **2a-e** were measured by the photon-counting method using luminol chemiluminescence as the standard.[8] The estimation of singlet excitation yields (Φ_S) was impossible because the emitter was not identified, but the relative value can be still presented as the products of the singlet excitation yield (Φ_S) and the fluorescence quantum yield (Φ_F) of the emitter, assuming that the emission arises from the same emitter in these chemiluminescence reactions. According to the equation, $\Phi_{CL} = \Phi_R \, \Phi_S \, \Phi_F$, the relative singlet excitation yield ($\Phi_S \cdot \Phi_F$) is calculated from the total

Table 1. Chemiluminescence quantum yield in the reaction of 9-phosphonoacridines (**2**) and alkaline hydrogen peroxide

| 9-phosphorylacridines	R	$\Phi_{CL} \times 10^9$ [a]	Φ_R [b]	$\Phi_S \cdot \Phi_F \times 10^9$
2a	OMe	1.6	0.82	2.0
2b	OEt	1.5	0.96	1.6
2c	OPr-*i*	1.5	0.98	1.5
2d	OPh	31.2	0.036	870
2e	Ph	120	0.92	130

[a] Determined by photon-counting method using luminol chemiluminescence as standard.
[b] Determined by UV spectrum.
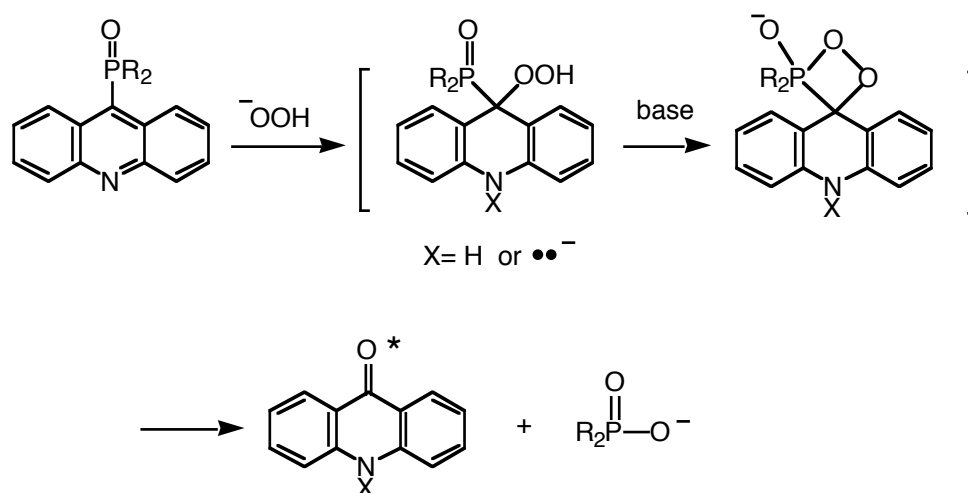
Scheme 2

chemiluminescence quantum yield (Φ_{CL}) and chemical yield (Φ_R) obtained by the measurements. These results are listed in Table 1. In spite of the much lower emission efficiency of the present chemiluminescence comparing to those for the 9-acridinecarboxylate,[9] a remarkable substituent effect occurred among these phosphorylacridines. Two orders of magnitude higher emission efficiencies were detected for **2d** and **2e** than for the other phosphonates (**2a-c**). Although having no distinct evidence yet, we believe that the excitation energy is produced during the ring cleavage of the phospha-1,2-dioxetanes generated by the oxy-Wittig-type reaction [10] as shown in Scheme 2. Some chemiluminescence reactions of the phosphonium ylides [11] and the phosphonate carbanions [7a, 12] are presumed to belong to the oxy-Wittig-type reaction. In this sense, the much larger value of $\Phi_S \cdot \Phi_F$ for **2e** is unexpected, because the oxy-Wittig-type reaction of **2e** seemed to be slow compared to the other phosphonates. The Wittig-type reaction of phosphine oxides sometimes did not produce the alkenes but the adducts still bearing the phosphoryl residues. [13] Furthermore, the formation of acridone in only a few percent yield from the reaction of **2d** means that the hydrolysis of the phosphorus groups chiefly led to the formation of a non-fluorescent compound, maybe an acridanylphosphonic acid, with liberation of phenol, namely, the nucleophilic attack of the peroxide ion to the phosphorus atom would be competitive with the attack on the 9-position of the acridine ring. Nevertheless, the relative Φ_S value of **2d** was much larger compared to the others. Such an observed substituent effect can be explained, provided the present chemiluminescence reaction involves the phospha-1,2-dioxetanes giving rise to a CIEEL process [14] as previously applied to the thermal decomposition of 10-methylacridane dioxetanes accompanied by light emission. [15] A remarkable substituent effect on the emission efficiency was documented in the chemiluminescence of 10-methylacridane dioxetanes, where the steric factor drastically controlled the emission efficiency. [15] Similar circumstances can be applied in the present phosphorylacridine system. The acridane ring is assumed to be in equilibrium between the *syn-* and *anti-*forms by a flutter as described in Scheme 3. Therefore, the distance between a nitrogen atom and O-O bond of the presumed phospha-1,2-dioxetne ring should be variable during the equilibrium.   Thus, the intramolecular electron transfer from the
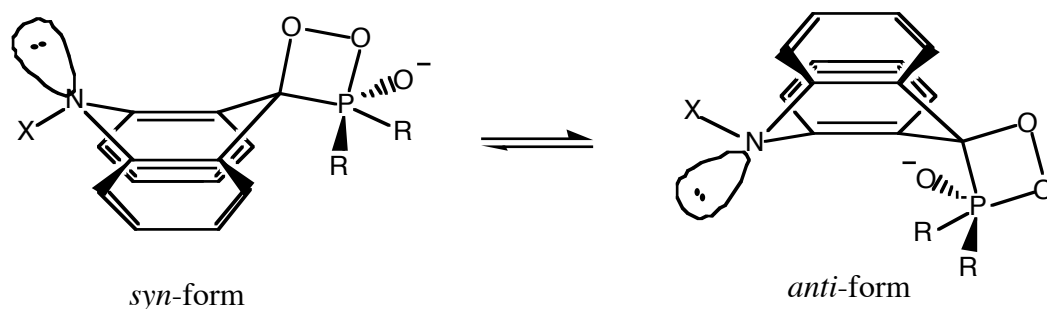
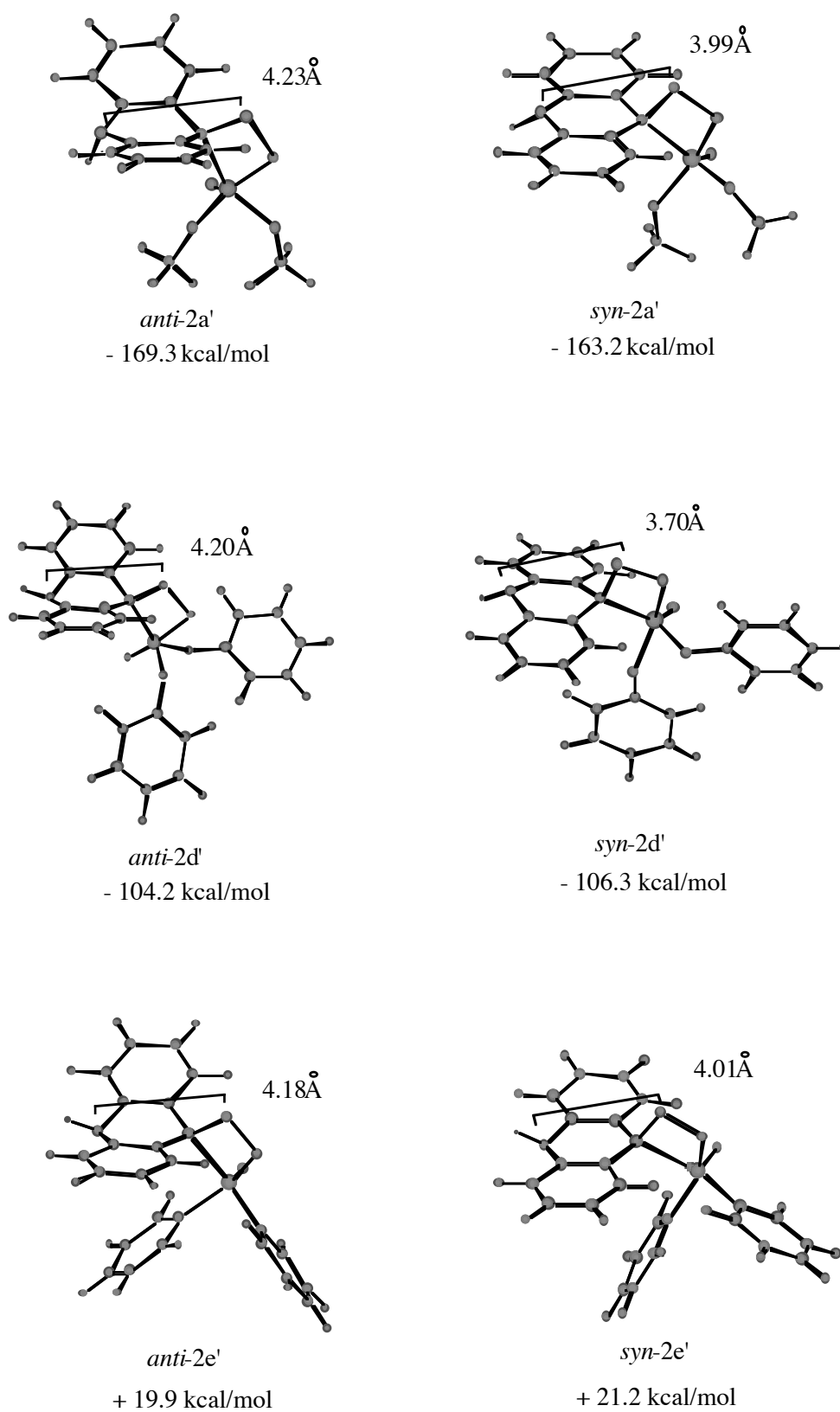*syn*-form   *anti*-form

Scheme 3

Figure 1. Structures and Heat Fomations (kcal/mol) of Presumed *anti-* and *syn*-Phospha-1,2-dioxetanes Calculated by the PM3 Method

nitrogen atom to the LUMO σ* O-O bond should be more advantageous in the *syn*-form compared to the *anti*-form. The bulky substituents attached to the acridane dioxetane ring would make the *syn*-conformer more advantageous than the other due to steric repulsion. The PM3 calculations were done to determine whether this assumption is appropriate. The conformers corresponding to the *anti*- and *syn*-forms derived from **2a**, **2d** and **2e** were located as depicted in Figure 1. Whereas *anti*-**2a'** is more stable than the *syn*-conformer, the energetic relation is inversed in **2d'**. Despite the higher energy of *syn*-**2e'** than *anti*-**2e'**, the energetic difference between both conformers is small compared to that of **2a**. Therefore, the assumption that the bulky substituents increase the ratio of the *syn*-form seems to be reasonable. As the distance between the nitrogen atom and the one side of the O-O bond in the *syn*-form is shorter than in the *anti*-from as shown in Figure 1, the intramolecular electron transfer in the *syn*-forms would be advantageous compared to the *anti*-forms.

White *et al*.[6] described that during the chemiluminescence of **1**, a dioxetanone intermediate was not always necessary but a transition state could also supply the energy enough to generate the excited acridone anion. Our results presented here do not completely exclude the role of the transition state bearing a close resemblance to the phospha-1,2-dioxetane in this chemiluminescence, but the substituent effect on the kinetics of the light emission observed in our preceding work of autoxidation of the phosphonate carbanions [7a] and the effect described in the present study from a steric point of view support the mechanism shown in Scheme 2.

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