the aldehyde proton in acrolein (no "13-methyl" group) and *cis*-crotonaldehyde can be seen, and the acrolein shift is substantially upfield. Comparison of the aldehyde proton chemical shifts for *trans*-crotonaldehyde and tiglaldehyde, lines 3 and 4 of Table III, shows that the effect of methyl substitution α to the aldehyde carbon is very small. In lines 5 and 7 can be seen the aldehyde chemical shifts of *all-trans*-retinal and *all-trans*-13-demethylretinal, showing that the effect of β-methyl substitution is the same in the conjugated polyene aldehydes as it is in the model compounds. The α–β single bond conformation for the compounds in lines 1, 3, and 5 has been shown to be planar *s*-trans, in the reference given in the last column of the table. Concerning the *cause* of the upfield shift accompanying the removal of an adjacent (β-) methyl group, it is at least partly explained as the removal of a source of steric polarization (a deshielding effect) of the aldehyde proton.[10,11]

Considering now the even more important C-10–C-13 region of 11-*cis*-13-demethylretinal, we note in Table I that the resonances of both 10-H and 13-H are considerably deshielded, relative to their positions in the trans isomer of this model chromophore. The shielding is the result of the kind of steric interaction[12] previously noted for the aldehyde proton. Interaction between 10-H and 13-H can occur in the 12-*s*-trans conformer of 11-*cis*-13-demethylretinal.

To confirm the structure and conformation of 11-*cis*-13-demethylretinal, several nuclear Overhauser experiments were performed.[13] When the 10-H resonance was saturated, a 17% enhancement was observed for 13-H, confirming the proximity of 10-H and 13-H in the molecular structure. When 13-H was irradiated, a 23% enhancement of 15-H was found; this enhancement is in close accord to the corresponding experiment on *trans*-crotonaldehyde in acetone-d_6 solution, where a 26% enhancement of the aldehyde proton resonance was found when the β proton was saturated.[9] The 15-H[13-H] result is indicative of a 14–15 planar *s*-trans conformation.

Nelson et al.[3] reported the presence of only two isomers when *all-trans*-13-demethylretinal was irradiated with visible light. These were separated chromatographically and tentatively identified as 9-cis and 11-cis isomers by their reaction with opsin. In view of the well-known difficulty of separating 11-cis from 13-cis isomers of retinal-like compounds (before the advent of high pressure liquid chromatography), the possibility existed that the isomer called *cis*-II, and identified as 11-cis, might actually be contaminated with 13-*cis*-demethylretinal. A photoisomerization experiment was done with a degassed dilute solution of the *all-trans*-13-demethylretinal compound irradiated continuously with light while held in a flat quartz cell. The resulting isomerate solution was transferred directly to an NMR sample tube and pulsed overnight. The spectra revealed considerable isomerization: 21% 9-cis, 4% 11-cis, and 75% all-trans was the isomeric composition of the product. There were no peaks in the isomerate spectrum which could be attributed to 13-*cis*-13-demethylretinal.[14] This is strikingly different photoisomerization behavior from that of normal *all-trans*-retinal, which under identical conditions yields 12% 9-cis, no detectable 11-cis, 20% 13-cis, and 60% all-trans.

Acknowledgments. The author is grateful to Professor A. Kropf of Amherst College for the very generous gift of the demethylretinals. The photochemistry was done in collaboration with Dan Rich and Professor Bryan Kohler with whom several helpful discussions are acknowledged. The NMR results were done in collaboration with Professor Brian Sykes. The financial support of the National Institutes of Health (Grant GM-17190 to B.D.S.) and the National Science Foundation (Predoctoral Fellowship to R.R.) are acknowledged.

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1,2-Dioxetane Formation in an Indole System[1]

Sir:

The cleavage of the 2,3 double bond of indoles by molecular oxygen has long been known in many chemical[2] and biological[3] oxygenations. In most cases 1,2-dioxetane has been assumed to be the precursor of the C_2–C_3 ring cleavage product,[2,4] while such a highly strained arene 1,2-dioxetane has not yet been proved experimentally. We now report the preparation and characterization of the 1,2-dioxetane in the low-temperature photooxygenation of *tert*-butylated indoles, the first example of the direct observation of the long-sought 1,2-dioxetane derived from indoles. We previously observed that singlet oxygenation of *N*-methylindoles results in the formation of the ring-cleavage products in high yield.[2a,6] In order to detect 1,2-dioxetane intermediates we have carried out the photooxygenations of a number of 2- and 2,3-substituted *N*-methylindoles at low temperature, and found that 2-*tert*-butyl-1,3-dimethylindole (**1**) gives a 1,2-dioxetane which has a long enough lifetime to allow chemical and spectroscopic characterization.

Photosensitized oxygenation of **1** (1.4 mmol) in $CFCl_3$ at −78 °C with tetraphenylporphyrin as sensitizer using a tungsten–bromine lamp gave rise to the formation of a peroxidic product which rapidly liberated iodine from aqueous alcohol solution of potassium iodide. Warming of the low-temperature solution to room temperature resulted in the formation of the ring-cleavage product **2** in a quantitative yield (Scheme I). The ^{1}H NMR spectrum ($CFCl_3$) of the low-temperature solution at −70 °C showed resonances at δ 1.22 (s, 9 H, *tert*-butyl), 2.01 (s, 3 H, *C*-methyl), 3.04 (s, 3 H, *N*-methyl), and 6.60–7.35 (m, 4 H, aromatic H).[7] The ^{1}H NMR signals completely disappeared within a few minute at 0 °C with the appearance of the peaks ascribable to **2**. The chemical shifts of the resonances of the unstable peroxide are inconsistent with the formulation as the zwitterion **3** which would be expected to have the *N*-methyl and *tert*-butyl resonances considerably downfield of the position of the spectrum, whereas the chemical shifts of the deshielded *C*-methyl protons[8] (δ 2.01) as well as *N*-methyl and *tert*-butyl protons are in the

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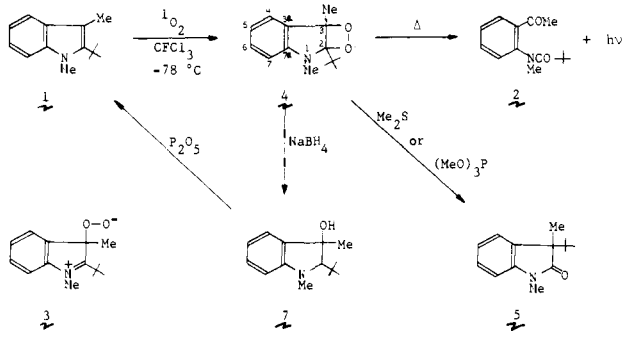
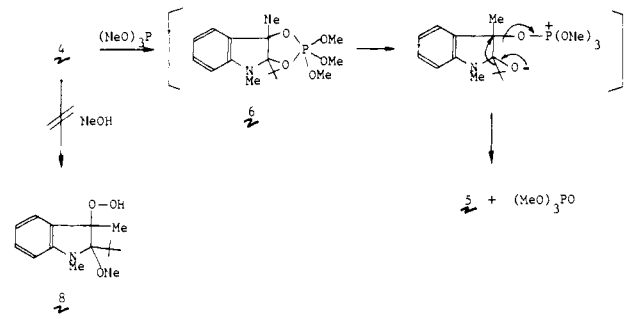
Scheme I



Scheme II



region reasonably expected for the 1,2-dioxetane (**4**). A set of four peaks in the aromatic region (δ 6.60–6.82 (2 H)) is also characteristic of the aromatic protons of the typical indoline derivatives.[6d,9] The ^{13}C NMR spectrum of the unstable peroxide is fully consistent with the monomeric 1,2-dioxetane (**4**). The spectrum at −70 °C showed two singlet peaks at δ 96.1 and 111.0 ascribable to the quaternary carbons of the dioxetane ring,[10] which disappeared rapidly on warming to 0 °C.

Further characterization was accomplished by reaction with reducing reagents. Reaction of **4** with dimethyl sulfide in methanol at −70 °C followed by the workup at room temperature gave 3-*tert*-butyl-1,3-dimethyl-2-indolinone[13] (**5**, 90%) and dimethyl sulfoxide.[14,15] Addition of an equimolar amount of trimethyl phosphite to the solution of **4** in $CFCl_3$ at −70 °C followed by monitoring the reaction by ^1H NMR at −70 °C resulted in the rapid and quantitative formation of **5** and trimethyl phosphate; an unstable phosphorane[16] such as **6** could not be detected under the conditions (Scheme II). Addition of the cold solution of **4** to precooled methanol (−70 °C) containing excess $NaBH_4$ followed by the workup at room temperature gave **7**[17] (60%), which on refluxing with phosphorus pentoxide in CCl_4 gave **1** (90%). Thermolysis of **4** in $CFCl_3$ at −10 °C resulted in a weak chemiluminescence with maximum around 450 nm, whereas the thermolysis in the presence of 9,10-dibromoanthracene (DBA) or 9,10-diphenylanthracene (DPA) gave an easily detectable chemiluminescence from the fluorescent singlet state of DBA or DPA detected at 430 nm.

The above spectroscopic and chemical evidence clearly indicates that the peroxidic product is the monomeric 1,2-dioxetane (**4**).[18] **4** decomposed instantaneously at 0 °C in most organic solvents yielding **2** as the only product; none of the trapping product such as **8** has been observed on the thermal decomposition of **4** in methanol.[19] The half-life of **4** at −20 °C measured by ^1H NMR at −70 °C is solvent dependent; in $CFCl_3$ **4** has a relatively long half-life (2700 s), whereas the rates of decomposition greatly increased in polar solvents such as CD_3COCD_3 (390 s) and CD_3COCD_3–CD_3CN (300 s).[20] Surprisingly, the half-life of **4** in CD_3OD is only 48 s at −20 °C.[22] In fact, low-temperature photooxygenation of **1** in methanol did not produce the 1,2-dioxetane (**4**) in an appreciable yield and gave the ring-cleavage product **2** as the major product.

Unlike **1**, low-temperature photooxygenation of other substituted *N*-methylindoles such as 1,3-dimethyl-, 1,2,3-trimethyl-, and 2-cyclopropyl-1,3-dimethylindoles did not produce the corresponding 1,2-dioxetanes detectable at −70 °C. Thus the introduction of a bulky *tert*-butyl group into the 2 position of indoles is an important factor for stabilizing the dioxetane ring. To our knowledge, this is a rare example of the direct observation of the 1,2-dioxetane in an aromatic system.[24]

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(7) The low-temperature solution contains **4** (93%) and a small amount (7%) of **2**. At −70 °C **2** exists as a 1:1 mixture of two rotational isomers: ^1H NMR ($CFCl_3$) δ 0.88 and 1.28 (s, *tert*-butyl), 2.41 and 2.54 (s, $COCH_3$), 3.01 and 3.46 (s, *N*-Me), 7.05–8.05 (m, aromatic H).
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(13) All new compounds except **4** gave satisfactory elemental analyses and mass spectral data. **5**: bp 68 °C (1 mmHg); ^1H NMR ($CDCl_3$) δ 1.00 (s, 9 H), 1.33 (s, 3 H), 3.17 (s, 3 H), 6.67–7.37 (m, 4 H); IR (Nujol) 1720 cm^{-1}; UV λ_{max} (EtOH) 256 nm (log ϵ 3.09), 277 (3.74).
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(17) **7**: mp 49–50 °C; ^1H NMR ($CDCl_3$) δ 1.17 (s, 9 H), 1.63 (s, 3 H), 1.70 (br s, 1 H, OH), 2.73 (s, 1 H), 2.93 (s, 3 H), 6.40–6.68 (m, 2 H), 7.02–7.20 (m, 2 H); UV (EtOH) λ_{max} 235 nm (log ϵ 3.50), 258 (3.82), 299 (3.30).
(18) Attempts to isolate a pure crystalline compound by column chromatography (silica gel or alumina) at −70 °C resulted in a rapid decomposition of **4**.
(19) The trapping product **8** has not been obtained also in the photooxygenation of **1** in methanol at −70 °C. See I. Saito, S. Matsugo, and T. Matsuura, submitted to *J. Am. Chem. Soc.* for publication.
(20) The solvent effect suggests an involvement of an intramolecular electron exchange process[21] in the decomposition of **4**. Mechanistic studies including solvent effect on the chemiluminescence of **4** are underway and will be discussed elsewhere.
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(22) This does not seem to be due to trace amounts of metal ions impurities contained in CD_3OD, since treated and untreated CD_3OD with disodium EDTA gave essentially the same result.[23]
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(24) So far several dioxetanes have been reported in the cycloaddition of singlet oxygen to aromatic compounds.[25] In contrast to the present case, all of the reported dioxetanes[26] have unusual thermal stability (mp >110 °C). The possibility that some of these dioxetanes may have other structures should not be overlooked.
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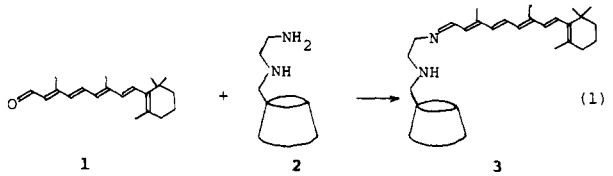
Cyclodextrin Having an Amino Group as a Rhodopsin Model

Sir:

Since the conception of the visual pigment was first provided by Hecht in 1920,[1] physicochemical aspects of the visual process have attracted the attention of many chemists. Although much important information about retinal **1** and rhodopsin (which is composed of retinal and receptor protein, opsin)[2] has been collected in numerous investigations, there still seems to be a serious gap or discrepancy between what is known about retinal itself and what is known about rhodopsin as the actual active pigment in biological systems. Among the significant discrepancies is that the large red shift in the electronic spectrum observed for the Schiff base of retinal bound to opsin has never been reproduced by retinal under any appropriate conditions in aqueous solution and several conflicting explanations for the observed discrepancy have been given.[3,4]

We now report the first successful binding model as an example of our current research using cyclodextrins as the recognition element.[5] We have substituted a ω-aminoethylamino group in place of one of the primary hydroxyl groups of β-cyclodextrin to give a simple binding model of rhodopsin. The resultant diamine, **2**, has a hydrophobic recognition site as well as a carbonyl recognition site to form the corresponding Schiff base. As expected, this host having double recognition sites binds retinal, a hydrophobic aldehyde, very strongly in aqueous solution. With regard to the present model it is interesting to note that the observed λ_{max} of its electronic spectrum is located at 497 nm, in excellent agreement with those of bovine rhodopsin (498 nm) or bovine lumirhodopsin (497 nm) (see Table I and Figure 1).

Thus, the Schiff base **3** was prepared from the reaction between 2 μmol of *all-trans*-retinal (**1**) and 17 μmol of ω-aminoethylamino-β-cyclodextrin (**2**)[6] in 0.5 mL of ethylene glycol at room temperature for 24 h in the dark (eq 1).[7] The corresponding open-chain Schiff bases, ω-retinylidene-*N*-methylethylenediamine (**4**) and ω-retinylidene-*n*-butylamine[8] (**5**)



1 2 3

Table I. Absorption Maxima of Retinal Schiff Bases at 25 °C

Schiff base	solvent	λ_{max} ($\Delta\lambda_{max}$)[a]
3	HO OH	375
3·$(H^+)_2$[b]	HO OH	476
3·$(H^+)_2$[c]	H_2O	497 (+21)
4	MeOH	367
4·$(H^+)_2$[b]	HO OH	470
4·$(H^+)_2$[c]	H_2O	471 (+1)
4·$(H^+)_2$ + β-CD[d]	H_2O	444 (−26)
5[e]	MeOH	366
5·H^+[b]	MeOH	450
5·H^+[c]	H_2O	450 (0)
5·H^+ + β-CD[d]	H_2O	433 (−17)
bovine rhodopsin[f]		498 (cis)
bovine lumirhodopsin[f,g]		497 (trans)

[a] Spectral shift of λ_{max} from that of each protonated species in methanol or ethylene glycol. [b] Excess of HCl was added. [c] At pH 1.16 in the aqueous HCl solution. [d] At pH 1.16 in the aqueous HCl solution, β-cyclodextrin, 2×10^{-2} mol/L. [e] I. Suzuki and Y. Kito, *Photochem. Photobiol.*, **15**, 275 (1972). [f] R. G. Mathews, R. Hubloard, P. K. Brown, and G. Wald, *J. Gen. Physiol.*, **47**, 215 (1963). [g] Lumirhodopsin is considered to contain *all-trans*-retinal as the chromophore. See ref 2f.

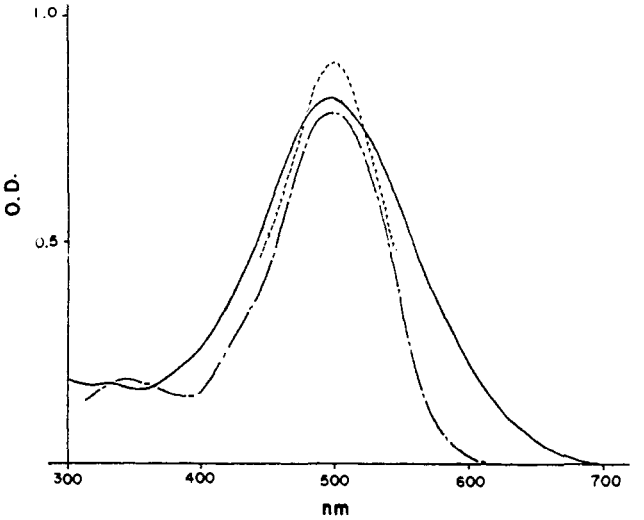
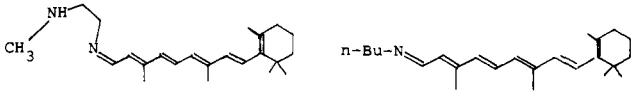


Figure 1. Electronic spectra of **3**·$(H^+)_2$ (—) at pH 1.16 in the aqueous HCl solution and bovin rhodopsin (- - -) and lumirhodopsin (- - - -) (G. Wald, J. Durell, and R. C. C. St. George, *Science*, **111**, 179 (1950)). Concentrations of all compounds are 2×10^{-5} M.

were also prepared by a similar procedure.

All of these Schiff bases, **3**–**5**, showed electronic spectra with "normal" λ_{max} at ~370 nm in methanol or ethylene glycol. In aqueous solution at pH 1, **4** and **5** were completely protonated



4 5

and their λ_{max}'s shifted to longer wavelength, independently of the solvent (water, methanol, or ethylene glycol). However, **3** behaved quite uniquely in aqueous solution at pH 1, although, on protonation in ethylene glycol, a red shift of a similar order of magnitude to that seen for **4**·$(H^+)_2$ was observed.[9] It is very

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