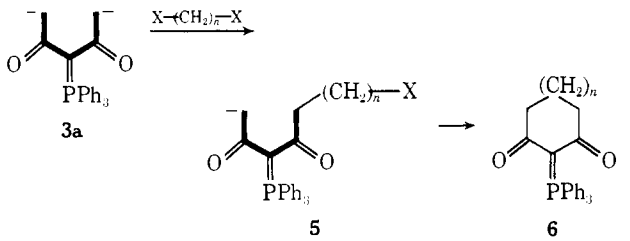
preference in these compounds in which the carbonyl oxygen adopts a cis orientation with respect to the phosphonium group. Variable temperature studies also indicate the presence of a high barrier to the carbon–carbon bond rotation required for the adoption of the trans conformation. In light of these results the presence of a single methyl resonance in the nmr spectrum of **1** suggests that this ylide exists in a "U" conformation[9] in which both oxygen atoms are cis to the phosphonium group in that the methyl groups present in the "sickle" conformation[9] would likely be nonequivalent.[10] In the absence of an appreciable alteration of the barrier to the bond rotations required for conformer–conformer interconversion, dianion **3**, derived from conformationally rigid **1**, would likewise possess the "U" conformation depicted in **3**, forcing a cis, proximal relationship between the nucleophilic centers. In this event dialkylation of **3a** with difunctional alkylating agents could result in ring formation owing to a favored intramolecular alkylation within the intermediate ylide anion **5**.

Attempts to effect such a ring closure by the alkylation of **3** with either methylene iodide or 1,2-dibromoethane failed to give identifiable products.[11] However, treatment of **3** (prepared from **1** and *n*-butyllithium) with 1,3-diiodopropane results in the formation of the desired cyclic ylide **6** (n = 3), mp 139–140°, isolated in



25% yield.[12,13] *Anal.* Calcd for $C_{26}H_{25}O_2P$: C, 77.98; H, 6.29. Found: C, 78.00; H, 6.12.

Evidence for the indicated structure of **6** (n = 3) is provided by its pmr spectrum ($CDCl_3$) which contains three broad resonance peaks at τ 2.39, 7.05, and 8.22 with an integral ratio of 15:4:6 representing phenyl, methylene adjacent to carbonyl, and remaining ring methylene protons, respectively. The infrared spectrum (KBr) of **6** (n = 3) contains carbonyl bands at 1590 and 1540 cm^{-1} comparable to the bands (1590, 1535 cm^{-1}) present in ylide **4** (R_1 = R_2 = Me). The presence of a molecular ion at *m/e* 400 in its mass spectrum further supports the proposed structure.

The unusual formation of an eight-membered ring by this type of intramolecular process, normally unfavorable in the formation of medium-sized rings, suggests great potential utility for the construction of difficultly obtained ring systems by the use of such conformationally restricted reagents. We are presently exploring the utility of this concept.

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Manning P. Cooke, Jr.,* Ramanuj Goswami
Department of Chemistry, Washington State University
Pullman, Washington 99163
Received August 13, 1973

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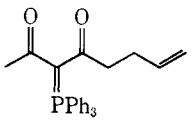
(10) The "W" conformation[9] would also allow methyl group equivalence but does not permit the cis oxygen–phosphonium group relationship suggested by chemical shift data.[8]

(11) The color of **3** is immediately discharged upon the addition of 1,2-dibromoethane suggesting that this reagent may be susceptible to a facile 1,2 debromination.

(12) No attempt has been made to optimize the yield or this reaction.

(13) When dianion **3** is prepared from **1** and lithium diisopropylamine and treated with 1,3-diiodopropane only a trace of cyclic ylide **5** is formed and i is observed to be the major product. This product is apparently the result of a base promoted dehydroiodination of either the alkylating agent or of the intermediate iodide **5** (n = 3, X = I). The intervention of such a process may be the result of the enhanced basicity of one of the anionic species in the presence of the diisopropylamine generated in the anion forming step. Such an effect has been observed with alkyllithium reagents in the presence of amines.[14]



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Fluorescence Characteristics of Phytochrome and Biliverdins

Sir:

Fluorescence of phytochrome (P_r) in solution has been reported by Hendricks, *et al.*,[1] and Correll, *et al.*[2] The latter workers obtained the fluorescence maximum of purified rye phytochrome in solution at 672 nm. More recently, however, highly purified and proteolytically undegraded phytochrome from rye showed no fluorescence in solution at room temperature.[3] The lack of fluorescence at room temperature has been confirmed in the present work. This communication describes low-temperature fluorescence characteristics of purified phytochrome and its model bilinoid pigments, biliverdin and biliverdin dimethyl ester.

Phytochrome was isolated from rye seedlings grown in the dark and was purified (absorbance at 280 nm/absorbance at 665 nm = 1.46) according to Tobin and Briggs.[3] Biliverdin (Sigma Chemical Co.) and biliverdin dimethyl ester (synthesized according to the literature[4]) were purified on various thin-layer chromatographic adsorbents. The best purification of the former was achieved by chromatography on polyamide layer with methanol–water (3:1), as described by Petryka and Watson.[5] Fluorescence spectra (uncorrected for detector response) were recorded on a high-resolution spectrometer with a single photon counting detector as described recently.[6]

Figure 1 shows the fluorescence spectrum of phytochrome at 14°K. In addition to the "normal" fluorescence maximum at 674.6 nm, there appears an unexpected fluorescence at 440 nm when the molecule is excited in the region of its second absorption band with

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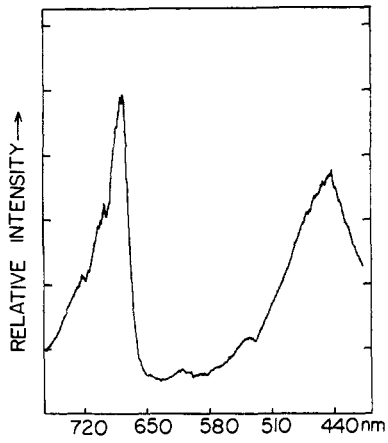

Figure 1. Fluorescence spectrum of phytochrome in glycerol–water (1:9, v/v) at 14°K. The excitation wavelength and bandpass were 380 and 6.4 nm, respectively, and the emission was recorded at 0.8-nm resolution.

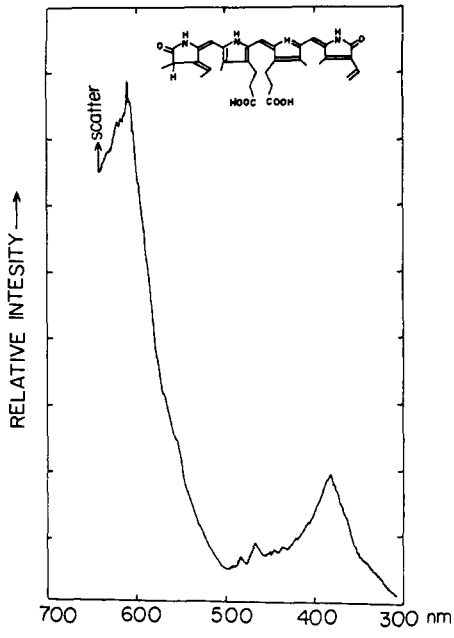

Figure 2. The fluorescence excitation spectrum of phytochrome in glycerol–water (1:9, v/v) at 14°K with emission monitored at 680 nm. The emission bandpass was 1.6 nm, and the excitation spectrum was recorded at 0.16-nm resolution. The tentative chromophore structure is inserted. The protein moiety is presumably bound to one of the carboxyl groups.

380-nm light. Both fluorescence bands are readily observable in the temperature range of 14–250°K, although their relative intensities vary with temperature. No fluorescence was observed at room temperature. (The detectability of the high-resolution spectrometer is $\Phi \geq 10^{-6}$, where Φ is the emission quantum yield.) Figure 2 shows the excitation spectrum of phytochrome, and it resembles the absorption spectrum, as expected.

Figure 3 shows the fluorescence spectrum of biliverdin at 77°K. Again a two-fluorescence band system is observed, as in the case of phytochrome. Figure 4 shows the low-temperature absorption spectrum which is closely matched by the excitation spectrum (not shown) with respect to the emission maximum at 725 nm. Biliverdin dimethyl ester shows essentially identical behavior.

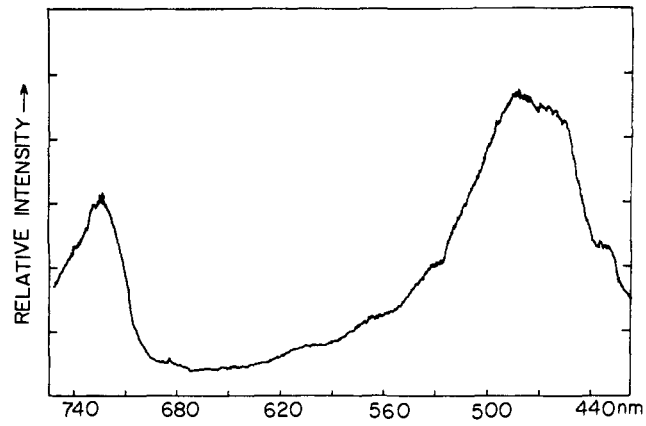

Figure 3. Fluorescence spectrum of biliverdin in ethanol at 77°K. The excitation wavelength and bandpass were 380 and 6.4 nm, and the emission spectrum was recorded at 0.64-nm resolution.

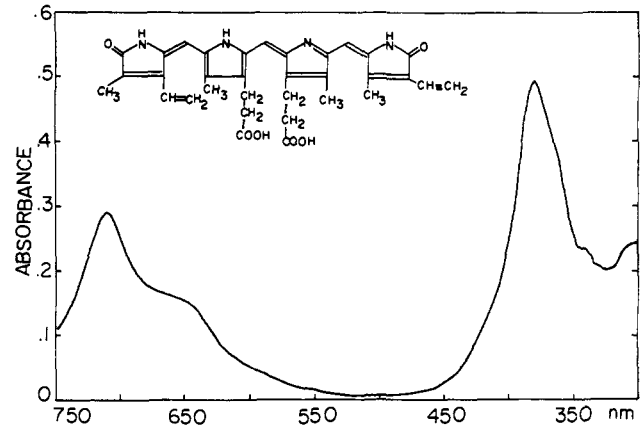

Figure 4. Absorption spectrum of biliverdin in ethanol at 77°K. The biliverdin structure is inserted.

The significance of the above results can be summarized as follows. First, the lack of fluorescence from highly purified phytochrome in solution at temperatures higher than 250°K implies the existence of a specific environment for the chromophore in which disposition of the excitation energy is efficiently coupled with the conformation of the interacting protein, possibly in a manner responsible for the phototransformation of the P_r form. The earlier reported fluorescence from phytochrome at room temperature may then be attributed to the emission from the chromophore with partially degraded (proteolytically) apoprotein or aggregates. Second, samples of phytochrome and two model chromophores (biliverdin and biliverdin dimethyl ester) show "anomalous" fluorescence when excited at the second absorption band of these compounds. It should be stressed that these compounds were highly purified and were obtained from three different sources, namely, rye seedlings, bovine gall, and chemical synthesis, respectively. It is, therefore, unlikely that the "anomalous" band is due to a nonbilinoid impurity. Luminescent impurities in solvents, optical cells, and optical artifacts have been excluded. We believe that the observed anomaly is an intrinsic property of some form of the bilinoid chromophores since many attempted purification procedures did not decrease the short-wavelength emission. We are currently investigating molecular mechanism(s) responsible

for this fluorescence using several other model compounds. Two working hypotheses concerning the anomalous fluorescence attribute it to (a) emission from the higher excited singlet state, analogous to the well known case of azulene,[7] and (b) conformational or tautomeric isomers which cannot be separated from the main components by chromatographic methods.[8] Photoproducts can be ruled out, since all steps of the experiments were carried out in the dark. The phytochrome spectrum was measured beginning at 14°K and up, so that the possibility of photoproduct formation can be ruled out, since at 14°K photoreactions are not likely to occur. Furthermore, biliverdins are very stable with respect to visible light.[9]

Regardless of the molecular mechanism for the short-wavelength fluorescence, the present results may have some bearing on the action spectra of the P_r–P_{fr} phototransformation and photomorphogenesis, particularly with respect to the actinic efficiency of the second absorption band of phytochrome at 380 nm.

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Pill-Soon Song,* Quae Chae, David A. Lightner
Department of Chemistry, Texas Tech University
Lubbock, Texas 79409

Winslow R. Briggs, David Hopkins
The Biological Laboratories, Harvard University
Cambridge, Massachusetts 02138

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Evidence for Oxirene Intermediates in the Peroxidation of Acetylenes

Sir:

Oxirenes, potential 4π antiaromatic systems,[1] have been frequently proposed as transient intermediates in the peroxyacid oxidation of acetylenes.[2-7] The results of the earlier product[2-6] and kinetic studies[7] are consistent with but do not uniquely require the oxirene mechanism. Recent evidence seems to favor a pathway featuring ketocarbenes as reactive intermediates.[8] We now present evidence which, for the first time, compellingly implicates oxirenes as the first-formed intermediates in the peroxidation of acetylenes. The conclusion is based on the observation (Figure 1) that a strikingly close quantitative correlation exists between the logarithmic rates of acetylene oxidation and those of the peroxidation of an olefin, both in a series of solvents of diverse nature. The assumed analogy to oxirane formation in the peroxidation of olefins, which formed the principal bases for the proposal of oxirene intermediacy in the peroxidation of acetylenes, is now firmly justified. The transient existence of oxirenes has also been demonstrated recently in the photochemical Wolff rearrangement,[9] the photolysis of ketenes,[10] and the reaction of methylene with carbon monoxide.[11]

Table I. Stoichiometry for Oxidation of 4-Octyne by MCPBA in Benzene Solvent

$[Octyne]_0$[a]	$[MCPBA]_0$[a]	[Octyne consumed][a,b]
0.100	0.100	0.064
0.080	0.040	0.022
0.120	0.040	0.032
0.160	0.040	0.041
0.200	0.040	0.040

[a] In mol $l.^{-1}$. [b] When MCPBA had reacted completely.

The oxidation of 4-octyne by *m*-chloroperoxybenzoic acid (MCPBA) has been studied. Exploratory gas chromatographic analysis of the reaction mixtures revealed a 1 to 1 stoichiometry of octyne to peroxyacid when the hydrocarbon was in at least fourfold excess over MCPBA. As shown in Table I, the observed stoichiometry changed progressively toward 1 mol octyne to 2 mol peroxyacid as the ratio of initial concentrations, $[octyne]_0/[MCPBA]_0$, was reduced step by step from 5 to 1.

The data of Table I show that under conditions where the stoichiometry is 1:1, the peroxyacid participates mainly in the primary oxidation step in which an oxygen atom is transferred to the acetylenic compound;[12] its involvement in secondary oxidations becomes significant when the ratio, $[octyne]_0/[MCPBA]_0$, is less than four.

The rates of oxidation of 4-octyne by MCPBA in a series of solvents, differing widely in polarity and structure, were followed iodometrically under conditions of 1 to 1 stoichiometry. The observed second-order rate constants[13] are listed in Table II along with the literature rate constants for the epoxidation of cyclohexene by peroxybenzoic acid (PBA).[14,15]

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(13) References 5 and 7 have shown that the peroxidation of acetylenes is governed by the second-order kinetic law. The present rate constants in the nonbasic solvents were calculated from the slopes of second-order plots which were linear to as far as the reaction was carried (usually 70–75%), while those in the basic solvents were calculated from initial rates. MCPBA decomposed in methanol, dioxane, and isopropyl alcohol solutions presumably due to trace metal catalyzed oxidations of these solvents. Dipicolinic acid ($\sim 10^{-3}$ *M*) was added for kinetic runs in these solvents which completely suppressed the decomposition of MCPBA in methanol and dioxane for more than the duration of kinetic runs. In isopropyl alcohol the trace metal catalysis could not be completely eliminated.
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