atoms in the normal alkyl chain of the substituent. Bis(*n*-alkylcyclohexano)-18-crown-6 compounds exhibited the same phenomenon. The longer substituents are believed to interfere with the approach of the substrate to the crown–metal salt complex.

The fluoride ion was extremely difficult to extract into the organic phase. The hydrated fluoride ion is apparently unable to penetrate the chloroform phase to allow complexation to occur. On the other hand, ligands that are water soluble enough to enter the aqueous phase are insufficiently lipophilic to draw the fluoride back into the organic phase so that reaction can occur. Nevertheless, the use of a liquid–liquid phase transfer system with a hard anion is impractical since solid–liquid systems have been found to perform satisfactorily with these types of anions.[31]

Crown ethers, no matter how modified, do not appear to be the catalysts of choice for phase-transfer catalysis. In liquid–liquid systems where the anion is soft, onium salts were found to be just as effective as the crown ethers and poly(ethylene glycol) was found to be satisfactory. Both of these are less expensive than crown ethers and are just as convenient to use. Although cryptand compounds were superior catalysts, they were not found to be cost effective since they cost 30–150 times that of the crown ethers. In solid–liquid systems, other researchers have already shown that crown ethers offer no particular advantage over onium salts when high dielectric constant solvents like acetonitrile are used.[18] Furthermore, glymes and poly(ethylene glycol) have also been shown to be effective, even in nonpolar solvents.[32-38]

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Formation and Hydrolysis of Lactones of Phenolic Acids

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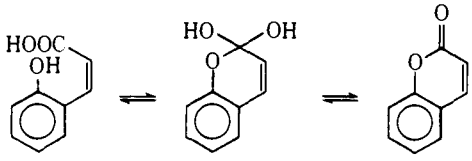
Abstract: The lactonization of 2′-hydroxybiphenyl-2-carboxylic acid to 3,4-benzocoumarin has been studied in aqueous solution, 30 °C, in the range of pH 0–6.5. As with simpler coumarinic acids, the rate-determining step changes from the breakdown of tetrahedral intermediates in the neutral or weakly acidic pH range to formation of intermediates below pH 2. In contrast, the lactonization of methyl-substituted dihydrocoumarinic acids shows no evidence for a change in rate-limiting step over a wide pH range. The hydrolysis of 8-hydroxy-1-naphthoic acid lactone in the range of pH 0–10 also does not undergo a transition in rate-limiting step. These results suggest that conjugation of the phenolic oxygen with the carbonyl carbon atom through an extended double-bond system may be necessary for the expulsion of water from a cationic tetrahedral intermediate and hence for the change in rate-determining step. Revision of the rate constants for the acid-catalyzed lactonization of dihydrocoumarinic acid and of 4,4,5,7-tetramethyldihydrocoumarinic acid indicates that the rate-enhancing effect of the "trimethyl lock" is significantly smaller than previously believed.

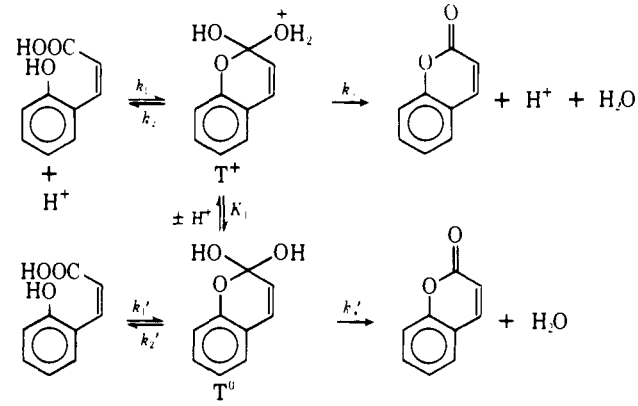
The lactonization of coumarinic acids proceeds via the formation of kinetically detectable tetrahedral addition intermediates (eq 1).[1,2] With most coumarinic acids, the rate-determining step for



(1)

lactonization changes from formation of the intermediate at low pH to breakdown of the intermediate at somewhat higher pH.[1-3] This behavior is believed to result from the different partitioning of the several ionic species of the tetrahedral intermediate between reactants and products (Scheme I). It is thought that the cationic intermediate T^+ expels mainly water ($k_3 \gg k_2$), while the neutral intermediate T^0 breaks down with predominant departure of phenol ($k_2' \gg k_3'$). In contrast, ^{18}O-exchange studies of the hydrolysis of phenyl acetate in 1.5 N HCl indicate that the ratio of the hydrolysis and exchange rates has a value of 120, so that

Scheme I



the rate of expulsion of phenol is considerably greater than that of water, even in fairly acidic medium.[4] In addition, the lactonization of the dihydrocoumarinic acid **3** (expressed in terms of the neutral acid) in acidic aqueous solution is reported to obey the simple rate law of eq 2 and thus shows no evidence for a change in rate-limiting step with changing pH.[5]

(1) (a) Garrett, E. R.; Lippold, B. C.; Mielck, J. C. *J. Pharm. Sci.* **1971**, *60*, 396–405. (b) Lippold, B. C.; Garrett, E. R. *Ibid.* **1971**, *60*, 1019–1027.
(2) (a) Hershfield, R.; Schmir, G. L. *J. Am. Chem. Soc.* **1973**, *95*, 7359–7369; (b) *Ibid.* **1973**, *95*, 8032–8040.
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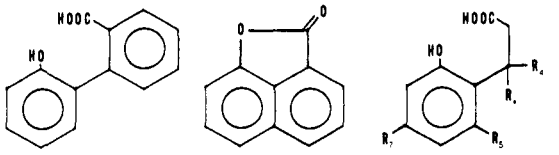

1 2 3 $R_4 = CH_3$ $R_5 = R_7 = H$; 4 $R_4 = R_5 = R_7 = CH_3$; 5 $R_4 = R_5 = R_7 = H$

$$k_{lac} = k_H[H^+] + k_{H_2O} \quad (2)$$

The factors which determine the direction of breakdown of the tetrahedral intermediates in the formation and hydrolysis of phenyl esters or lactones are not completely understood at present. The research described here was undertaken to ascertain the importance of two such factors in promoting the expulsion of water from the tetrahedral intermediate: (a) the linkage of the phenolic hydroxyl group to the carbonyl carbon via conjugated double bonds, and (b) the existence of a relatively high equilibrium constant in favor of lactone formation.

The following reactions were selected for detailed study: (a) the lactonization of 2'-hydroxybiphenyl-2-carboxylic acid (**1**) to 3,4-benzocoumarin, (b) the hydrolysis of the lactone of 8-hydroxy-1-naphthoic acid (**2**), and (c) the lactonization of two dihydrocoumarinic acids (**3** and **4**)[33] to the corresponding dihydrocoumarins. Hydroxy acid **1** may be considered a coumarinic acid analogue whose side-chain double bond has been incorporated into an additional aromatic system. With lactone **2**, extended resonance structures involving both the phenolic oxygen and the carbonyl group cannot be written. The hydroxy acids **3** and **4** lack the side-chain double bond of coumarinic acids but possess methyl substituents which drive the hydroxy acid–lactone equilibrium far in favor of the lactone.[6]

Results

The rate of lactonization of **1** at 30 °C in 0.7% ethanol–water (μ = 1.0, maintained with added LiCl) was determined in the range of pH 0–6.5. At pH >3, the reactions were carried out in unbuffered solution, and the pH was kept constant by automatic titration with a pH-stat.[2b,7]

As with simpler coumarinic acids,[1,2] the first-order rate constants for the lactonization of **1** decrease steadily with increasing pH and reach a constant value at pH 5–6 (Table Ia[8] and Figure 1A). After correction for the ionization of the carboxyl group (eq 3),

$$\frac{k_{obsd}}{f} = \frac{k_{obsd}}{[H^+]/([H^+] + K_a)} \quad (3)$$

the rate of the lactonization expressed in terms of neutral reactant still exhibits the complex dependence on pH usually found with coumarinic acids (Figure 1B). Use was made of the mechanism outlined in Scheme I and the derived steady-state rate eq 4 to

$$\frac{k_{obsd}}{f} = \frac{k_1([H^+]P^+ + K'P^0)(H^+ + K'(1 - P^0)/(1 - P^+))}{[H^+] + K'} \quad (4)$$

interpret the pH–rate profile for the lactonization of **1**. The rate constant k_{OH} for the hydroxide ion catalysis of lactonization at pH >5 was obtained from the slope ($k_{OH}K_w$) of the linear plot of k_{obsd}/f vs. $1/[H^+]$. After subtraction of the contribution of the hydroxide-dependent term from the overall rate, the remaining pH–rate profile was fitted to eq 4 by a nonlinear least-squares procedure. The constants P^+ and P^0 are the partitioning ratios of intermediates T^+ and T^0, respectively, while pK' is equal to the pH value where the change in rate-determining step takes place (see Table II, footnote f). The last independently determined constant obtained is k_1; the values of the other constants (Table II) which may be calculated from the pH–rate profile of **1** are fixed by the values of P^+, P^0, K', and k_1.[2a]

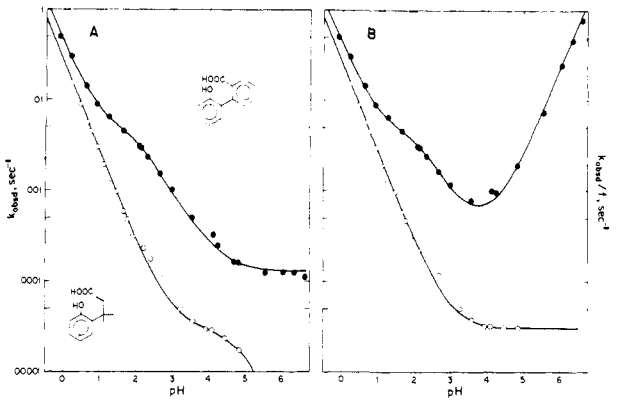

Figure 1. (A) pH–rate profiles for lactonization of **1** (●) and **3** (○). For **1**, the line is calculated from eq 4, to which the contribution of the base-catalyzed lactonization has been added and the resulting rate constants multiplied by the mole fraction of **1** in the neutral form. For **3**, the line is calculated from eq 6. Constants used are from Table II. (B) pH–rate profiles expressed in terms of neutral substrate. Lines are calculated from eq 4 for **1** (●) and from eq 7 for **3** (○).

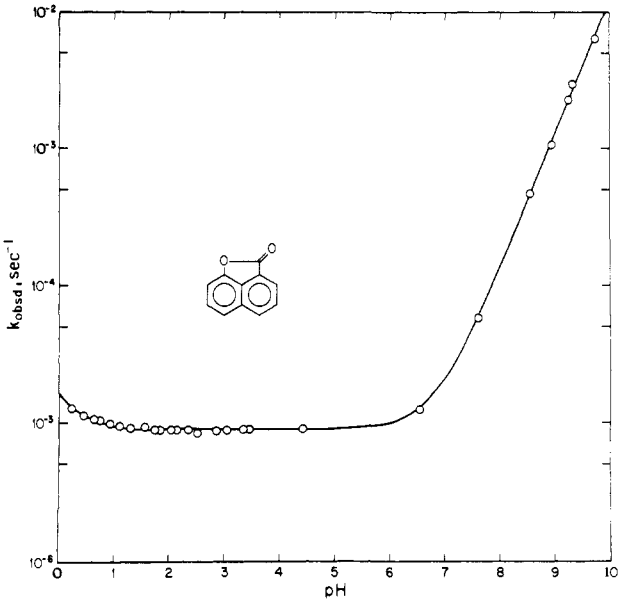

Figure 2. pH–rate profile for hydrolysis of **2**. Line is calculated from eq 5, using constants of Table II.

The effect of varying pH on the rate of hydrolysis of lactone **2** (1% acetonitrile–water, μ = 0.5, 30 °C) is shown in Figure 2. The rate data (Table Ib)[8] follow the simple rate law of eq 5, and

$$k_{obsd} = k_H[H^+] + k_{H_2O} + k_{OH}K_w/[H^+] \quad (5)$$

the derived constants are given in Table II. Since all rate measurements were performed on solutions of constant ionic strength, the modest rate increase below pH 1 (40% at the highest acidity used) probably represents real acid catalysis, though there have been reports that the substitution of H^+ for Li^+ at constant ionic strength may result in either a small increase[9] or decrease[10] in rate, owing to activity coefficient effects. The results of previous studies of the hydrolysis of **2** are in reasonable agreement with those of the present work. Values for k_{OH} of 71 M^{-1} s^{-1} (in 1.7%

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(7) Bruice, T. C.; Maley, R. *Anal. Biochem.* **1970**, *34*, 275–278.
(8) See the paragraph at the end of the paper regarding supplementary material.
(9) (a) Marburg, S.; Jencks, W. P. *J. Am. Chem. Soc.* **1962**, *84*, 232–239. (b) Bruice, T. C.; Holmquist, B. *Ibid.* **1967**, *89*, 4028–4034.
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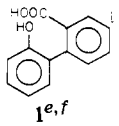
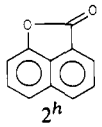
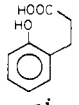
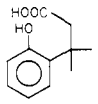
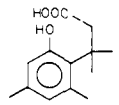
Table II. Rate and Equilibrium Constants for Hydroxy Acid Lactonization and Lactone Hydrolysis

	lactonization			lactone hydrolysis			equilibrium constant[a]		pK_a	
	k_H, M^{-1} s^{-1}	k_{H_2O}, s^{-1}	k_{OH},[b] M^{-1} s^{-1}	k_H, M^{-1} s^{-1}	k_{H_2O}, s^{-1}	k_{OH},[b] M^{-1} s^{-1}	spectral[c]	kinetic[d]	RCOOH	phenol
 1[e,f]	0.76	4.5×10^{-4}	6.7×10^5[g]			12[g]	4.8×10^4	5.6×10^4	3.78	10.33
 2[h]				7.5×10^{-6}	8.9×10^{-6}	82[i]				
 5[j]	(7×10^{-5})[k]			8.5×10^{-3}			8.2×10^{-3}			
 3	2.7×10^{-2}[l] 2.8×10^{-2}[j]	3×10^{-5}[l] 1.2×10^{-5}[j]				4.2[l,q]	390[l] 280[j]		4.95[l] 5.69[j] 7.38[m]	
 4	2.5[m] 26.2[j]	1.1×10^{-4}[m] 7.5×10^{-3}[j]	4.8×10^3[m,n] 2.6×10^5[j,o]			0.048[m,n] 0.4[j,o]	6.0×10^5[j]	10^5[m] 6.5×10^5[j]	7.38[m] 5.78[j,p]	12.67[j]

[a] K_{eq} = [lactone]/[neutral hydroxy acid]. [b] Based on activity of OH^-, calculated from measured pH and appropriate value of pK_w (indicated in each case). [c] Calculated from UV absorbance of reaction at equilibrium. [d] Calculated from rate constants for forward and reverse reactions. [e] 30 °C, 0.7% ethanol-water, μ = 1.0 (LiCl). [f] The rate constants given in the table are for lactonization with rate-limiting breakdown of intermediates. For rate-limiting formation of intermediates, $k_1 = 0.042$ M^{-1} s^{-1}, $k_1' = 4.96 \times 10^{-3}$ s^{-1}. Other constants calculated from Figure 1 are $P^+ = 0.953 \pm 0.002$, $P^0 = 0.090 \pm 0.003$, and $pK' = 2.21 \pm 0.04$, where $P^+ = k_3/(k_2 + k_3)$, $P^0 = k_3'/(k_2' + k_3')$, and $K' = K_1(k_2' + k_3')/(k_2 + k_3)$; note that constants for rate-determining breakdown of intermediates may be expressed as $k_H = k_1P^+(1 - P^0)/(1 - P^+)$ and $k_{H_2O} = k_1'P^0$.[2a] All constants refer to Scheme I. [g] Based on $pK_w = 13.50$. [h] 30 °C, 1% acetonitrile-water, μ = 0.5 (LiCl). [i] Based on $pK_w = 13.83$. [j] 30 °C, 20% dioxane-water, μ = 0.3 (NaCl). [k] Calculated from equilibrium constant and rate constant for reverse reaction. [l] 30 °C, 1% ethanol-water, μ = 0.5 (LiCl). [m] 20 °C, 60% dimethoxyethane-water, μ = 0.1 (LiCl). [n] Based on $pK_w = 15.04$. [o] Based on $pK_w = 14.09$. [p] Estimated from kinetic data (Figure 3). [q] Based on $pK_w = 13.90$.

acetonitrile–water, μ = 0.5, 25 °C)[11] and 147 M^{-1} s^{-1} (in 30% dioxane–water, μ = 1.0, 29.9 °C)[12] and for k_{H_2O} of 9.8×10^{-6} s^{-1} (30% dioxane–water, μ = 1.0, 20 °C)[12] have been reported. The straightforward pH–rate profile for the hydrolysis of **2** in the range of pH 0–10 provides no information concerning the possible participation of intermediates in this reaction.

Although rate data for the lactonization of the dihydrocoumarinic acid **3** have been published,[5] a detailed reinvestigation of this reaction was undertaken in an attempt to detect a possible change in rate-determining step with varying pH. Rate constants (Table Ic)[8] for lactonization in 1% ethanol–water, μ = 0.5 (LiCl), 30 °C, were obtained over the range of pH 0–5. As reported earlier,[5] general acid–base catalysis by buffer components is observed, and first-order rate constants were extrapolated to zero buffer concentration to provide the buffer-independent pH–rate profile (Figure 1A). The kinetics of the conversion of **3** to the corresponding dihydrocoumarin are satisfactorily represented by eq 6, which is based on the assumption that the lactonization of

$$k_{obsd} = (k_H[H^+] + k_{H_2O})([H^+]/([H^+] + K_a)) \quad (6)$$

the neutral acid is subject to catalysis by hydronium ion and water. The pH–rate profile corrected for carboxyl group ionization (eq 7) exhibits none of the complexity shown by the corresponding

$$k_{obsd}/f = k_H[H^+] + k_{H_2O} \quad (7)$$

plot for **1** (Figure 1B). The values of k_H and k_{H_2O} (Table II) are very close to those ($k_H = 2.72 \times 10^{-2}$ M^{-1} s^{-1}; $k_{H_2O} = 3.63 \times 10^{-5}$ s^{-1}) previously determined[5] under nearly identical conditions (except that ionic strength was maintained at 0.3 M). Values for k_H and k_{H_2O} obtained from a limited study of the lactonization of **3** in 20% dioxane–H_2O (30 °C, μ = 0.3, pH 2.14–2.76) are also given in Table II.

The buffer-independent pH–rate profile for the lactonization of **4** (in 20% dioxane–water, μ = 0.3 with added NaCl, 30 °C) follows eq 8 in the range of pH 2–11 (Figure 3). As in the case

$$k_{obsd} = (k_H[H^+] + k_{H_2O} + k_{OH}K_w/[H^+])([H^+]/([H^+] + K_a)) \quad (8)$$

of **3**, correction of k_{obsd} for the ionization of the carboxyl group simplifies the appearance of the profile which is readily shown to consist of the sum of terms for acid, water, and base catalysis of the lactonization reaction (eq 5). Owing to the rapidity of the formation of the dihydrocoumarin, the pK_a of the carboxyl group of **4** could not be easily measured by titration. The value of pK_a = 5.78, which was estimated from the fit of the data in Figure 3 to eq 8, may be compared to the value of 5.69 which was obtained by titration of **3** under the same conditions. That the ionizable groups of hydroxy acid **3** may be used as models for those of **4** is supported by the observation that both compounds have a carboxyl pK of 7.38 in 60% dimethoxyethane, a solvent in which the rate of lactonization of **4** has been sufficiently reduced so that the carboxyl pK_a can be measured by ordinary acid–base titration.

Observed first-order rate constants for the lactonization of **4** are given in Table Id[8] and the derived constants of eq 8 are summarized in Table II. Substantial differences exist between the values of the rate constants determined in this study and those previously reported[6] for this compound studied under identical conditions of solvent, temperature, and ionic strength. The largest

(11) Menger, F. M.; Brock, H. T. *Tetrahedron* **1968**, *24*, 3453–3459.
(12) Bowden, K.; Law, D.; Ranson, R. J. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1799–1804.

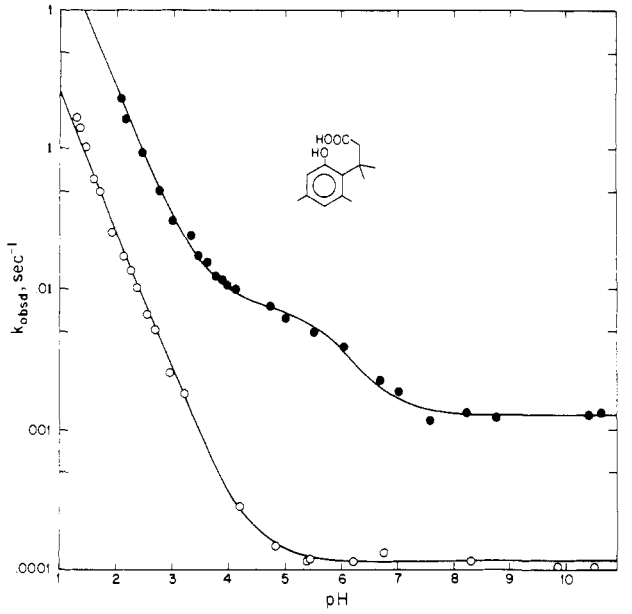


Figure 3. pH–rate profile for lactonization of **4**. (●) 30 °C, 20% dioxane–water; (○) 20 °C, 60% dimethoxyethane–water. Lines are calculated from eq 8, using constants of Table II.

difference occurs in k_H, where the present value of 26 M^{-1} s^{-1} is almost 20 000 times smaller than the published value of 5×10^5 M^{-1} s^{-1}.[13]

The lactonization of **4** in 60% dimethoxyethane–water (μ = 0.1, 20 °C) occurs more slowly than in 20% dioxane–water, and its pH–rate profile (Table Ie[8] and Figure 3) shows no break resulting from the ionization of the carboxylic acid function of the reactant. This curious feature results from the fortuitous circumstance that eq 9 holds under these conditions. Using pK_a

$$k_{H_2O} = k_{OH}K_w/K_a \qquad (9)$$

= 7.38 and pK_w = 15.04 leads to a calculated value of 4.6×10^7 M^{-1} for the ratio k_{OH}/k_{H_2O} in 60% dimethoxyethane. For the reaction in 20% dioxane, eq 9 does not apply. Individual values for k_{H_2O} and k_{OH} are obtained from the appropriate sections of the pH–rate profile, and their ratio is 3.5×10^7 M^{-1}. The general agreement for this ratio in the two solvent systems lends support for this interpretation of the "masking" of the carboxyl ionization in the kinetics of lactonization in 60% dimethoxyethane.

Second-order rate constants (Tables If[8] and II) for the alkaline hydrolysis of the lactones formed from hydroxy acids **1**, **3**, and **4** were obtained from the linear dependence of k_{obsd} on hydroxide ion activity.

Equilibrium constants, expressed in terms of neutral hydroxy acid going to lactone, were determined in two ways. With **1**, **3**, and **4**, the concentration of neutral acid present at equilibrium at low pH cannot easily be measured, since lactone formation is enormously favored. However, the equilibrium can be shifted in the direction of the hydroxy acid simply by increasing pH sufficiently to ionize the carboxyl group and, if necessary, the phenolic hydroxyl group.[1,14] The pH-independent equilibrium constant is obtained from the apparent equilibrium constant K_{eq}' (eq 10a)

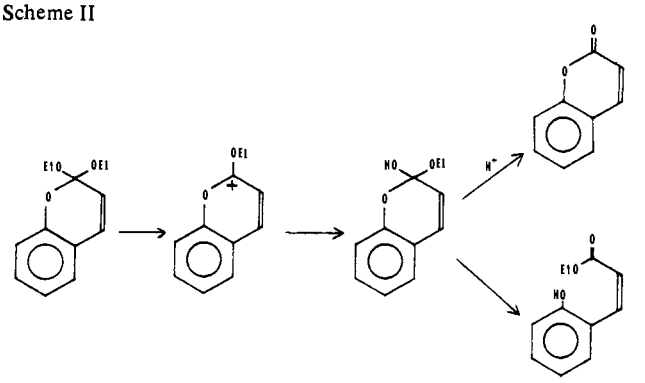
$$K_{eq}' = \frac{[\text{lactone}]_{eq}}{[\text{hydroxy acid}_{total}]_{eq}} \qquad (10a)$$

$$K_{eq} = K_{eq}'(1 + K_a/[H^+] + K_aK_b/[H^+]^2) \qquad (10b)$$

(13) The kinetics of the lactonization of hydroxy acid **4** and of other derivatives of 4,4,5-trimethyldihydrocoumarinic acid are being reexamined by Cohen and co-workers, and their findings will be published separately. Preliminary results with **4** are in essential agreement with our data (Cohen, L. A., personal communication).

(14) For another example of the use of this method to calculate equilibrium constants in lactonization reactions, see: Storm, D. R.; Koshland, D. E. *J. Am. Chem. Soc.* **1972**, *94*, 5805–5814.

Scheme II



by use of eq 10b, where K_a and K_b are the dissociation constants of the carboxyl and phenolic groups, respectively. Values of K_{eq} for the lactonization of **1**, **3**, and **4** are recorded in Table II. In two instances (**1** and **4**), the equilibrium constant was also calculated from the ratio of the rate constant for base-catalyzed lactonization to that for base-catalyzed hydrolysis of the lactone. This kinetic method, based on the assumption that these two rate constants refer to the same rate-determining step, was used[2] with an extensive series of coumarinic acids. Its validity is affirmed by the reasonable agreement found here between the values of K_{eq} obtained by the two approaches (Table II). Previous measurements had given a value of 25.67 for the lactonization of **3**, and an estimate of >99 for 4,4,5,7,8-pentamethyldihydrocoumarinic acid (both in 20% dioxane–H_2O).[6b]

The rate constant for the acid-catalyzed conversion of **5** to the unsubstituted lactone dihydrocoumarin is necessary to evaluate the rate enhancement produced by the "trimethyl lock"[6] in **4**. The rate constant for lactonization is best obtained from the equilibrium constant and the rate constant for the reverse reaction,[6] owing to the small extent of conversion of hydroxy acid to lactone at equilibrium.[15a] Values of 1.65×10^{-4} M^{-1} s^{-1} (30 °C, 20% dioxane–H_2O, μ = 0.3 (NaCl))[6b] and 42.2×10^{-4} M^{-1} s^{-1} (25 °C, 1 M $HClO_4$)[15b] have been reported for the acid-catalyzed hydrolysis of dihydrocoumarin. Since this difference seemed too large to be accounted for by a solvent or salt effect, the hydrolysis of dihydrocoumarin in 20% dioxane–water, μ = 0.3, 30 °C, was studied in dilute HCl solution (0.05–0.3 N), yielding $k_H = 85 \times 10^{-4}$ M^{-1} s^{-1} (Table Ig).[8] Spectrophotometric determination of the equilibrium constant in the same solvent gave $K_{eq} = 8.2 \times 10^{-3}$, which is smaller than the published value[6] of 37.3×10^{-3}. The rate constant for the acid-catalyzed lactonization of dihydrocoumarinic acid is therefore 7.0×10^{-5} M^{-1} s^{-1}.

Discussion

In addition to the lactonization of coumarinic acids,[1,2] there has been reported to our knowledge only one other reaction where the tetrahedral intermediate of ester formation (or hydrolysis) partitions differently at different pH. A transition in rate-determining step occurs in 1.15 M $HClO_4$ in the hydrolysis of ethyl trichloroacetate.[16] At higher acidity, the intermediate expels only ethanol, while the predominant leaving group at lower acidity is water. These conclusions are supported by measurements of the extent of oxygen exchange which accompanies hydrolysis of this ester.[16b] With the coumarinic acids, the transition in the rate-limiting step takes place at pH 0.3–4.0, depending on substituents.[1,2] The suggestion[2] that water is preferentially expelled from a tetrahedral intermediate at pH values below the transition pH while phenol departs at higher pH values is confirmed by a recent study of the hydrolysis of coumarin diethyl acetal (Scheme II).[3] The observation that coumarin is the main product of hydrolysis

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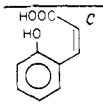
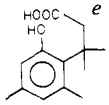
Table III. Comparison of Rate and Equilibrium Constants for Lactonization of Coumarinic and 4,4,5,7-Tetramethyldihydrocoumarinic Acids

compd	k_H,[a] $M^{-1} s^{-1}$	k_{H_2O},[a] s^{-1}	k_{OH},[a] $M^{-1} s^{-1}$	k_{OH},[b] $M^{-1} s^{-1}$	K_{eq}
 [c]	4.3	4.3×10^{-4}	1.8×10^{6} [d]	1.3[d]	14×10^{5}
 [e]	26.2	7.5×10^{-3}	2.6×10^{5} [f]	0.4[f]	6.2×10^{5}

[a] Lactonization. [b] Lactone hydrolysis. [c] Data taken from ref 2a; 30 °C, 0.1% ethanol-water, $\mu = 1.0$. For lactonization, rate constants are for rate-limiting breakdown of intermediates. [d] Based on $pK_w = 13.83$. [e] 30 °C, 20% dioxane-water, $\mu = 0.3$. [f] Based on $pK_w = 14.09$.

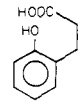
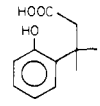
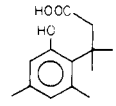
in 0.1–1.0 M HCl while coumarinic acid ethyl ester is the initial hydrolysis product in less acidic solution is entirely consistent with expectation if the hemiacetal intermediate in Scheme II is a reasonable model for the tetrahedral intermediate of Scheme I.

The rate and equilibrium constants for the lactonization of **1** are similar to those previously reported for a series of coumarinic acids,[2a] though the "substituent effect" of the ring fused to the coumarinic acid side chain in **1** is not expressed in a completely regular manner in the various constants. For example, while the partitioning ratio P^+ of the cationic intermediate and the pH where the change in rate-limiting step occurs ($pK' = 2.20$) are close to those of coumarinic acids with weakly electron-withdrawing substituents, the overall equilibrium constant is smaller than expected on this basis and is similar to those for the lactonization of coumarinic acids with strongly electron-withdrawing groups.

Although the presence of a side-chain double bond seems to be essential for the expulsion of water from the intermediate and the consequent change in rate-determining step (see below), neither the incorporation of the double bond in an extended resonating system such as in **1** nor the presence of strongly electron-withdrawing substituents on the double bond itself (3-chloro-, 3-bromo-, or 3-phenylcoumarinic acid)[1b,2a] has a profound effect on the mechanism of lactonization. The absence of a kinetically detectable change in rate-limiting step in the hydrolysis[17a] of the lactone **2** points to the need for conjugative interaction between the phenolic hydroxyl group and the carboxyl carbon, possibly to stabilize the oxocarbonium ion formed by loss of water from a cationic tetrahedral intermediate. Since phenol is expected to be a better leaving group than water under most circumstances and also since the ester C–O single bond which links 1,8-naphthalene positions may be strained,[17b,c] the rate-limiting step in the hydrolysis of **2** is probably the addition of water or hydroxide ion to the carbonyl group.[12] This likely occurrence of strain in **2** may be responsible (at least in part) for the favored departure of phenol from a putative cationic tetrahedral intermediate, so that the lack of a change in the rate-limiting step is probably the result of strain as well as electronic factors.

The requirement for the extended conjugated system of the coumarinic acids to permit the departure of water is further underscored by the failure of the dihydrocoumarinic acids **3** and **4** to undergo a change in rate-determining step as pH is decreased. On the basis of substituent effects, it has been concluded that the lactonization of **3** and its 6-substituted derivatives occurs with rate-limiting breakdown of tetrahedral intermediates.[2a,5] There is no reason at present to suspect that the lactonization of the more highly substituted dihydrocoumarinic acid **4** proceeds by a mechanism different from that of **3**. It seems, therefore, that the thermodynamic stability of the reaction product suggested by an equilibrium constant highly favorable to lactone formation may be necessary but not sufficient to induce partitioning of T^+ with expulsion of water rather than phenol.[18]

Table IV. Rate and Equilibrium Constants for Lactonization of Dihydrocoumarinic Acids[a]

			
k_H, $M^{-1} s^{-1}$	7.0×10^{-5}	2.8×10^{-2}	26.2
k_{rel}	1	400	3.7×10^{5}
K_{eq}[b]	8.2×10^{-3}	280	6.2×10^{5}
K_{rel}	1	3.4×10^{4}	7.6×10^{7}

[a] 30 °C, 20% dioxane-water, $\mu = 0.3$. [b] K_{eq} = [lactone]/[neutral acid].

The rate and equilibrium constants for the lactonization of **4** are generally similar to those for coumarinic acid[2a] (Table III). Nevertheless, while the pH–rate profile for coumarinic acid[2a] clearly shows the negative deviations from a line of slope −1 which signal the approaching change in rate-limiting step at pH 2.6, the corresponding plot for **4** remains linear down to pH 2 in 20% dioxane and down to pH 1 in 60% dimethoxyethane. Therefore, if a change in the rate-determining step occurs in the lactonization of **4** (and there is no evidence at all for it), it must take place at considerably greater acidity. The apparent similarity of these two lactonization reactions which is suggested by the resemblance of the kinetic constants at pH >3 gives few clues to the basis for the striking difference in behavior at lower pH.

Previous reports[6] that conformational restrictions may lead to rate enhancements of up to 10^{11} in the lactonization of dihydrocoumarinic acids have attracted much attention and engendered considerable speculation concerning the possible sources for these extraordinary rate increases.[19–25] The revised values of rate and equilibrium constants (Table IV) indicate that the rate enhancements resulting from the "trimethyl lock" are of the order of 10^5 which is, though by no means a negligible factor, less dramatic than previously believed and is more in line with rate increases observed in related systems.[26] The difficulties encountered[21,24] in attempts to account quantitatively for rate increases of 10^{11} thus find a simple resolution.

(17) (a) If a change in the rate-determining step is expected to occur in lactonization, it must also take place in the reverse reaction, owing to the requirements of the principle of microscopic reversibility. The value of the equilibrium constant for the overall reaction determines whether it is more convenient to study the formation or the hydrolysis of the lactone. Although the value of the equilibrium constant for the interconversion of **2** and the corresponding hydroxy acid is not known, the equilibrium seems to overwhelmingly favor the hydroxy acid,[17c] even at pH values low enough to keep the carboxyl group in the neutral form. (b) Balasubramaniyan, V. *Chem. Rev.* **1966**, *66*, 567–641. (c) Packer, R. J.; Smith, D. C. C. *J. Chem. Soc. C.* **1967**, 2194–2201.

(18) It has been proposed[19–22] that the hydroxy acid **4** is severely strained as a result of nonbonded interactions between the methyl groups in positions 4 and 5, so that a high equilibrium constant in favor of lactone formation may not reflect intrinsic thermodynamic stability of the lactone so much as relief of ground-state strain in the hydroxy acid. These suggestions, however, were made in attempts to account for a rate enhancement of 10^{11} when the rate of lactonization of **4** is compared to that of the unsubstituted dihydrocoumarinic acid **5**. In view of the present findings that the rate enhancement is in fact much smaller, the question of the importance of strain in the ground state of **4** for the rate of its lactonization remains open.

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Table V. Experimental Conditions for Formation and Hydrolysis of Lactones

compd	solvent	substrate concn, M	stock solution	wavelength,[a] nm	A^b (HCl concn, M)	B^b (NaOH concn, M)	pK_w[c]
1	0.7% EtOH-H_2O, μ = 1.0 (LiCl)	10^{-4}	lactone 0.03 M, NaOH 0.07 M, 30% EtOH	270	−0.10 (0.001-0.1)	13.40 (0.01-0.05)	13.50
2	1% MeCN-H_2O, μ = 0.5 (LiCl)	1.5×10^{-4}		337	−0.07 (0.002-0.1)		13.83[d]
3	1% EtOH-H_2O, μ = 0.5 (LiCl)	10^{-4}	lactone 0.03 M, NaOH 0.07 M, 75% EtOH	272	−0.07 (0.002-0.1)	13.83 (0.01-0.05)	13.90
4	60% dimethoxyethane-H_2O, μ = 0.1 (LiCl)	3×10^{-4}	lactone 0.1 M, NaOH 0.5 M, 50% dimethoxyethane	286[e] 300[f]	0.23 (0.001-0.1)	15.27 (0.07-0.1)	15.04
	20% dioxane-H_2O, μ = 0.3 (NaCl)	3×10^{-4}	lactone 0.1 M, NaOH 0.5 M, 50% dioxane	286[e] 300[f]	0.0 (0.002-0.1)	14.09 (0.001-0.1)	14.09
5	20% dioxane-H_2O, μ = 0.3 (NaCl)	3×10^{-4}		272			

[a] Wavelength at which lactonization or lactone hydrolysis was followed. Where only one number is given, both reactions were followed at the same wavelength. [b] Value of constant A in eq 11 and of constant B in eq 12. The term in parentheses gives the range of HCl or NaOH concentrations used to determine the values of A and B. [c] Ion product of water, calculated as in ref 12. [d] Taken from H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions", 3rd ed., Reinhold, New York, 1958, p 645. [e] Lactonization. [f] Lactone hydrolysis.

Experimental Section[27]

Acetonitrile[28] and dioxane[29] were purified according to published procedures. 1,2-Dimethoxyethane was distilled from sodium ribbon and then from lithium aluminum hydride. Dihydrocoumarin (Aldrich) had bp 112 °C (2.5 mm). Other organic liquids were distilled before use. Buffers and inorganic salts were reagent grade and were used without further purification. Freshly boiled, deionized, glass-distilled water was used for all aqueous preparations. 8-Hydroxy-1-naphthoic acid lactone (**2**), prepared according to Birch et al.,[30] was purified by sublimation: mp 104–105 °C (lit. mp 99–101,[30] 103–104,[12] 106–107 °C[11]); IR (Nujol) 5.54 μm (C=O); UV (1% CH_3CN-H_2O) λ_{max} 337 nm (ϵ_{max} 6250). 3,4-Benzocoumarin[31] was recrystallized from cyclohexane: mp 92.5–93 °C (lit.[31] mp 93–94 °C); IR (Nujol) 5.77 μm (C=O). 4,4-Dimethyldihydrocoumarin[5] had bp 106–108 °C (1.0 mm) (lit.[5] mp 81–83 °C (0.15 mm)); IR (neat) 5.75 μm (C=O); NMR ($CDCl_3$) δ 1.32 (6.1 H, $C(CH_3)_2$), 2.58 (1.9 H, CH_2). 4,4,5,7-Tetramethyldihydrocoumarin[6b] had mp 93–94 °C (lit.[6b] mp 90–92 °C); IR (Nujol) 5.64 μm (C=O); NMR ($CDCl_3$) 1.40 (6.1 H, $C(CH_3)_2$), 2.23 (3.0 H, CH_3), 2.43 (3.0 H, CH_3), 2.55 (2.0 H, CH_2), 6.70 (2.0 H, CH).

Kinetic Studies. The rates of formation or hydrolysis of the lactones were determined by following the changes in UV absorbance caused by the disappearance of reactants or appearance of products. A Cary Model 15 spectrophotometer equipped with an automatic cell-changer assembly was used. Values of pH were measured with a Radiometer TTTlc pH meter with a scale expander. Constant temperature was maintained by circulating water from a Haake constant-temperature bath through the jacketed cell holder of the Cary or through the jacketed glass vessel used for pH measurments. Reactions were initiated by adding 10–30 μL of the stock solution of hydroxy acid (salt) or lactone to 3 mL of buffer which had been allowed to reach constant temperature in the Cary cell holder. With **1** at pH >3, pH was kept constant by means of a Radiometer TTTla pH-stat, and the course of the reaction was followed spectrophotometrically. The contents of the quartz cuvette were rapidly mixed by vigorous shaking, after which continuous recording of the absorbance changes was begun. Stock solutions of the hydroxy acids were prepared by alkaline hydrolysis of concentrated solutions of the lactones in mixed aqueous–organic solvents. Specific details for each compound studied are given in Table V.

The compositions of the solvents used and the nature of the salts employed to maintain constant ionic strength are listed in Table V. All reactions were carried out at 30 °C, with the exception of the lactonization of **4** in 60% dimethoxyethane, which was studied at 20 °C. Buffers used were HCl, formate, acetate, phosphate, *N*-methylmorpholine, borate, carbonate, imidazole, Tris, triethylamine, and NaOH, in the appropriate ranges. With the exception of HCl solutions, buffer concentrations did not in general exceed 0.1 M.

The fourth column in Table V describes the conditions under which the stock solutions of the hydroxy acid sodium salts were prepared from the corresponding lactones. Stock solutions of the lactones needed for the studies of lactone hydrolysis were made up in pure ethanol, acetonitrile, dimethoxyethane, or dioxane, as appropriate.

Linear relationships between measured pH and the stoichiometric concentration of HCl (eq 11) or NaOH (eq 12) were found to hold in

$$\text{pH} = -\log (\text{HCl concentration}) + A \quad (11)$$

$$\text{pH} = \log (\text{NaOH concentration}) + B \quad (12)$$

dilute solutions of HCl and NaOH. These equations, together with the values of A and B given in Table V (columns 6 and 7), were used to calculate pH for more concentrated solutions of HCl or NaOH and also to calculate pK_w[12] for each of the solvents used in this study.

Calculation of first-order rate constants for the hydrolysis of **2** at pH <7.6 was done by following the reactions for at least 2 half-lives and by employing a modified Guggenheim treatment.[32] All other reactions were followed to completion (>8 half-lives), and rate constants were obtained from the slopes of semilogarithmic plots of absorbance changes vs. time by means of a linear least-squares program.

pK_a Determinations. With the exception of 60% dimethoxyethane solvent, all pK_a measurements were carried out under conditions of solvent, temperature, and ionic strength identical with those used in the kinetic studies. Solutions of the salts of the hydroxy acids were prepared by alkaline hydrolysis of the corresponding lactones in ethanol–water or dioxane–water. This procedure could not be used when the solvent was dimethoxyethane, because exposure of this solvent to aqueous alkali produced some unknown titratable substance which consumed HCl during the titration. Although this impurity was present in very small amounts, it interfered with the titration of the hydroxy acid carboxylate function. This difficulty was circumvented by performing the alkaline hydrolysis of the lactone in aqueous ethanol. An aliquot of this solution was then added to 60% dimethoxyethane–water which contained enough HCl so that the pH of the final mixture was <9.5. Therefore, the solvent for this titration was 60% dimethoxyethane–0.8% ethanol–water.

pK_a values for the carboxyl group of **3** (0.001 M) and of **4** (0.002 M) were determined by potentiometric titration. Spectrophotometric titration[2a] at 362 nm was used to obtain both pK_a values of **1** (0.0003 M) and at 286 nm for the phenolic pK of **4** (0.0007 M).

Equilibrium Constants. Buffered solutions of hydroxy acids or lactones were kept at 30 °C until the lactone–hydroxy acid equilibrium was established, and apparent equilibrium constants (K_{eq}') were calculated by using the known molar extinction coefficients of the various species present at equilibrium.

1: Absorbance was measured at 302 nm where ϵ for lactone, neutral hydroxy acid, monoanion, and dianion are 3530, 2690, 950, and 4730, respectively. Starting with lactone at pH 8.33, 8.59, and 8.77 gave values for K_{eq}' of 1.2, 0.61, and 0.39, respectively. Starting with hydroxy acid at pH 8.42 gave $K_{eq}' = 1.5$.

(27) Melting points and boiling points are uncorrected. NMR spectra were obtained with a Varian T-60 spectrometer and are given in parts per million relative to internal tetramethylsilane. IR spectra were recorded with a Perkin-Elmer 137 spectrometer.

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(33) The chroman numbering system employed by Milstien and Cohen[6b] has also been used in this paper.

3: (a) 1% Ethanol–H_2O. Absorbance was measured at 272 nm where ϵ for lactone and hydroxy acid monoanion are 470 and 1830, respectively. Starting with lactone at pH 6.92 and 7.22 gave K_{eq}' = 4.3 and 2.0, respectively. Starting with hydroxy acid at pH 6.95 and 7.28 gave K_{eq}' = 3.9 and 1.9, respectively. (b) 20% Dioxane–H_2O. Absorbance was measured at 278 nm where ϵ for lactone and hydroxy acid are 74 and 1750, respectively. Starting with hydroxy acid at pH 8.35, 8.51, 8.69, and 8.90 gave K_{eq}' = 0.63, 0.40, 0.29, and 0.17, respectively.

4: Absorbance was measured at 286 nm, where ϵ for lactone, monoanion, and dianion are 20, 1390 and 2300, respectively. Starting with lactone at pH 10.99, 11.40, 11.42, and 11.97 gave K_{eq}' values of 3.4, 1.4, 1.4, and 0.30, respectively. The pK_a value of 5.78 (carboxyl group) estimated from the pH–rate profile for the lactonization of **4** was used to calculate the value of the pH-independent equilibrium constant.

5: Although the mole fraction of dihydrocoumarin present in equilibrium with **5** is very small, the large difference in molar extinction coefficient between lactone (ϵ_{240} = 2140) and hydroxy acid (ϵ_{240} = 100) allows a reasonably accurate measurement of K_{eq}. The absorbance increased from 0.56 to 0.65, yielding $K_{eq} = 8.2 \times 10^{-3}$, starting with **5** at 5.6×10^{-3} M (0.1 M HCl). A second experiment with **5** at 1.15×10^{-2} M gave an absorbance change from 1.154 to 1.350, from which $K_{eq} = 8.3 \times 10^{-3}$ was obtained.

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Supplementary Material Available: Tables of observed first-order rate constants for formation and hydrolysis of lactones (Tables Ia–Ig) (13 pages). Ordering information is given on any current masthead page.

Chromopeptides from Phytochrome. The Structure and Linkage of the P_R Form of the Phytochrome Chromophore

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Abstract: The isolation and chromatographic purification of chromophore-containing peptides from the P_R form of phytochrome treated with pepsin and thermolysin are described. From the amino acid sequence and ^1H NMR spectral analysis of phytochromobiliundecapeptide (**2**), the structure of the P_R phytochrome chromophore and the nature of the thioether linkage joining pigment to peptide have been established. Confirmatory evidence was obtained from similar analysis of phytochromobilioctapeptide (**3**). The implications of this structural assignment with respect to the mechanism of the P_R to P_{FR} phototransformation are considered.

Owing to the wide range of light-controlled development and metabolic processes in green plants believed to be mediated by phytochrome, this biliprotein has been exhaustively studied by plant physiologists for many years.[1] Phytochrome has also received extensive study by physical and biological chemists because it exists in two spectrally distinct forms P_R (λ_{max} 665 nm) and P_{FR} (λ_{max} 720 nm) which are interconvertible upon absorption of light.[2] Despite the tremendous interest in this unusual photoreceptor, neither the chemical structure nor the precise nature of the chromophore–protein linkage of the P_R or P_{FR} chromophore has been definitively established.[2]

The numerous structures proposed for the phytochrome chromophore have been based primarily on degradative approaches which have involved spectroscopic analyses of altered forms of the chromophore, released from phytochrome after treatment with refluxing methanol[3] or with chromic acid.[4] In contrast to these previous studies of the phytochrome chromophore, our approach is based on the chromophore as well as the chromophore–protein linkage remaining unchanged throughout the analysis. Previously we have successfully applied this methodology to the structure elucidation of the β_1-phycocyanobiliheptapeptide (**1**) isolated from *C*-phycocyanin.[5] Now we provide ^1H NMR spectroscopic evidence for the structure and linkage of the P_R form of the phytochrome chromophore.

In 1971 Fry and Mumford partially determined the amino acid sequence of a phytochromobiliundecapeptide isolated from "small" oat phytochrome treated with pepsin.[6] In the present investigation, we describe the isolation of phytochromobiliundecapeptide (**2**) and phytochromobilioctapeptide (**3**) following the sequential pepsin–thermolysin digestion of oat phytochrome in the P_R form. ^1H NMR spectra were obtained, and their analyses provided proof of the structure and thioether linkage of the P_R form of the phytochrome chromophore.

Results and Discussion

Phytochrome Purification. The routine isolation of 50–60 mg of brushite-purified oat phytochrome with a specific absorption ratio (SAR = A_{667nm}/A_{280nm}) of 0.07[2a] from 4 kg-batches of etiolated oat seedlings was accomplished as described.[7] Crude phytochrome fractions eluted from brushite chromatography were assayed by measuring the double-difference spectra with a modified Cary 118 spectrometer.[8] As shown in Figure 1, this low-purity phytochrome was phototransformable, although a dramatic increase in turbidity accompanied the P_R to P_{FR} conversion.

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