Hydration of the Flavylium Ion. 3.[1,2] The Effect of 3-Alkyl Substitution

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An investigation is reported of the transformations undergone by four flavylium salts—two 3-methyl-substituted cations, the 3-methylflavylium ion, and the 4'-methoxy-3-methylflavylium ion—and two cations where an ethylene unit bridges the 3-position and the 2'-position of the phenyl ring, the 5,6-dihydrobenzo[c]xanthylium ion, and the 3-methoxy-5,6-dihydrobenzo[c]xanthylium ion. Three transformations have been identified and studied—a rapid and reversible hydration of the cations to produce a pseudobase, the rapid reversible ring opening of this pseudobase to a (Z)-chalcone, and the slow irreversible cis–trans isomerization of this species to an (E)-chalcone. Rate and equilibrium constants have been obtained for these processes and compared with values previously obtained for 3-H-substituted flavylium ions, the parent flavylium ion itself, and the 4'-methoxyflavylium ion. There is little effect of the alkyl substitution on the pseudobase: (Z)-chalcone equilibration. The ethylene-bridged systems undergo cis–trans isomerization at a similar rate to the 3-H systems, while the 3-methyl systems undergo this reaction about 30 times more slowly. The most significant effect, however, is seen on the flavylium ion hydration equilibrium, the bridged cations being about 2 orders of magnitude more stable than their 3-H-substituted counterparts with these in turn 2 orders of magnitude more stable than the 3-methyl analogues. To explain this, it is proposed that there is a steric interaction involving the 3-hydrogen or 3-methyl substituent and an ortho hydrogen on the nearby phenyl ring, resulting in a twisting of this ring so that it is not coplanar with the benzopyrylium portion of the cation.

Flavylium ions (F^+) are 2-aryl-substituted benzopyrylium cations of biological importance as the basic ring structure of anthocyanins, the pigments responsible for a variety of plant and flower colors.[3] Both synthetic and naturally occurring cations are stable only in relatively acidic aqueous solutions, with various structural changes occurring at higher pH. These transformations have been examined in varying degrees of detail for a number of systems, ranging from natural plant material to simple models.[4] We have recently reported studies of the parent flavylium ion itself,[1] and some simple 4'-substituted derivatives (4'-Me,[1] 4'-MeO,[1] and 4'-OH[2]), identifying various intermediates or products and establishing kinetic and thermodynamic relationships. In this paper we report an investigation of two 3-methyl-substituted flavylium cations (3-MeF$^+$ and 4'-MeO-3-MeF$^+$). These were chosen in order to investigate the effect of 3-substitution, since naturally occurring anthocyanins are invariably oxygen substituted at this position.[5] Also included in this report are the 5,6-dihydrobenzo[c]xanthylium cations **2** and **3**. These are flavylium ion derivatives where the 3-position is joined to the 2-aryl ring by an ethylene bridge.

Experimental Section

Materials. The perchlorate salts of the flavylium ions were synthesized via acid condensation of salicylaldehyde and the appropriate aromatic ketone,[6] with two recrystallizations from 10% perchloric acid/acetic acid.

F$^+$

2, X = H
3, X = OCH$_3$

3-Methylflavylium perchlorate: mp 177–178 °C; UV (1 M HCl), λ_{max} 387 nm (ϵ 2.8 × 10^4). Anal. Calcd for C$_{15}$H$_{13}$ClO$_5$: C, 59.92; H, 4.08; Cl, 11.05. Found: C, 60.11; H, 4.00; Cl, 10.91.

4'-Methoxy-3-methylflavylium perchlorate: mp 188–189.5 °C; UV (1 M HCl), λ_{max} 430 nm (ϵ 3.2 × 10^4). Anal. Calcd for C$_{16}$H$_{15}$ClO$_6$: C, 58.21; H, 4.30; Cl, 10.10. Found: C, 58.21; H, 4.12; Cl, 10.06.

5,6-Dihydrobenzo[c]xanthylium perchlorate: mp 193.5–195 °C; UV (0.1 M HCl), λ_{max} 420 nm (ϵ 3.4 × 10^4). Anal. Calcd for C$_{16}$H$_{13}$ClO$_5$: C, 61.37; H, 3.94; Cl, 10.65. Found: C, 61.61; H, 3.86; Cl, 10.81.

3-Methoxy-5,6-dihydrobenzo[c]xanthylium perchlorate: mp 237–238 °C; UV (0.1 M HCl), λ_{max} 452 nm (ϵ 4.4 × 10^4). Anal. Calcd for C$_{17}$H$_{15}$ClO$_6$: C, 59.60; H, 4.16; Cl, 9.77. Found: C, 59.40; H, 4.22; Cl, 10.00.

(E)-3,4-Dihydro-2-((2-hydroxyphenyl)methylene)-1(2H)-naphthalenone was prepared by the base-catalyzed aldol condensation of salicyladehyde and α-tetralone,[7] with recrystallization from absolute alcohol/ether. The product had a melting point of 147–148.5 °C. Anal. Calcd for C$_{16}$H$_{14}$O$_2$: C, 81.58; H, 5.64. Found: C, 81.60; H, 5.41. The E geometry about the carbon-carbon double bond is established by the observation that this product does not form the 5,6-dihydrobenzo[c]xanthylium ion in acid solutions.

Kinetics and Equilibrium Measurements. These were carried out essentially as previously described,[1] with the exception that with the less stable 3-methylflavylium ions a stock solution 0.05 M in dry acetonitrile was prepared and added to appropriate aqueous solutions.

Results

The behaviors of the flavylium ions of this study are, not unexpectedly, qualitatively similar to that exhibited by the parent flavylium ion, and the experimental approach and analysis therefore follows the same lines as that presented in detail previously.[1] The following sections

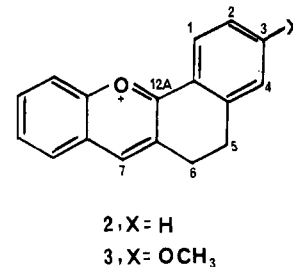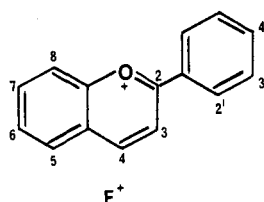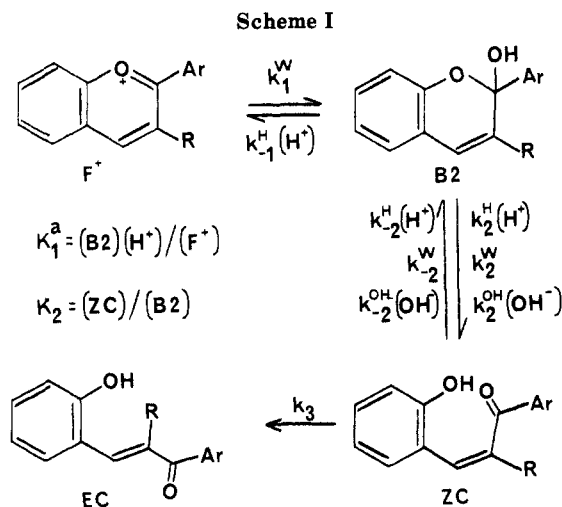(1) Part 1: McClelland, R. A.; Gedge, S. *J. Am. Chem. Soc.* 1980, *102*, 5838.

(2) Part 2: McClelland, R. A.; McGall, G. *J. Org. Chem.* 1982, *47*, 3730.

(3) (a) Hayashi, K. In "The Chemistry of Flavanoid Compounds"; Geissman, T. A., Ed.; Pergamon Press: Oxford, 1962; p 248. (b) Jurd, L. In "The Chemistry of Plant Pigments"; Chichester, C. O., Ed.; Academic Press: New York, 1972; p 123. (c) Jurd, L. *Recent Adv. Phytochem.* 1972, 135.

(4) (a) Sondheimer, E. *J. Am. Chem. Soc.* 1953, *75*, 1507. (b) Jurd, L.; Horowitz, R. M. *J. Org. Chem.* 1961, *26*, 2561. (c) Jurd, L. *J. Org. Chem.* 1963, *28*, 987. (d) Jurd, L. *J. Food Sci.* 1964, *29*, 16. (e) Timberlake, C. F.; Bridle, P. *Nature (London)* 1966, *212*, 518. (f) Harper, K. A.; Chandler, B. V. *Aust. J. Chem.* 1967, *20*, 731. (g) Nilson, E. *Acta Chem. Scand.* 1967, *21*, 1942. (h) Jurd, L. *Tetrahedron* 1969, *23*, 2367. (i) Brouillard, R.; Dubois, J. E. *J. Am. Chem. Soc.* 1977, *99*, 1359. (j) Brouillard, R.; Delaporte, B. *J. Am. Chem. Soc.* 1977, *99*, 8461. (k) Brouillard, R.; Delaporte, B.; Dubois, J. E. *J. Am. Chem. Soc.* 1978, *100*, 6202.

(5) (a) Interestingly the 3-methoxyflavylium ion is not stable even in 1 M HCl.[5b] This would obviously be a better model, but this instability makes a detailed study difficult since we require an acid solution which is not too concentrated where the cation is stable. (b) McGall, G.; McClelland, R. A., unpublished observation.

(6) Michaeldis, C.; Wizinger, R. *Helv. Chim. Acta* 1951, *34*, 1761.

(7) Jurd, L. *Tetrahedron* 1969, *23*, 2379.

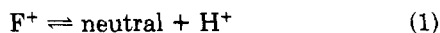**Table I. Rate Constants and Equilibrium Constants for the Transformations of Flavylium Ions (25 °C)**

const	F$^+$	4′-MeOF$^+$	3-MeF$^+$	4′-MeO-3-MeF$^+$	BX$^+$	3-MeOBX$^+$
λ_{max} (cation)	397	440	387	430	420	452
p$K_{obsd}{}^a$ (F$^+$)	2.98	4.29	1.22	2.28	4.90	6.39
p$K_1{}^a$	3.01	4.47	1.22	2.36	4.94	6.58
$k_1{}^W$, s^{-1}	4.59	0.47	17.7	3.4	0.87	0.04
$k_{-1}{}^H$, M^{-1} s^{-1}	4.7 × 10^3	1.4 × 10^4	3.0 × 10^2	7.7 × 10^2	7.6 × 10^4	1.4 × 10^5
K_2	0.06	0.50	~0.02^b	0.2	0.09	0.56
$k_2{}^H$, M^{-1} s^{-1}	2.5	19.5	c	24	0.53	2.1
$k_2{}^W$, s^{-1}	0.11	0.23	c	0.08	0.02	0.063
$k_{-2}{}^H$, M^{-1} s^{-1}	42	39	c	120	5.9	3.7
$k_{-2}{}^W$, s^{-1}	1.8	0.46	c	0.4	0.23	0.11
k_3, s^{-1}	2.6 × 10^{-4}	4.6 × 10^{-5}	9 × 10^{-6}	d	1.3 × 10^{-4}	d

^{a} F$^+$, flavylium; 4′-MeOF$^+$, 4′-methoxyflavylium; 3-MeF$^+$, 3-methylflavylium; 4′-MeO-3-MeF$^+$, 4′-methoxy-3-methylflavylium; BX$^+$, 5,6-dihydrobenzo[c]xanthylium; 3-MeOBX$^+$, 3-methoxy-5,6-dihydrobenzo[c]xanthylium. ^{b} Estimated. ^{c} Could not be measured. ^{d} Not determined.
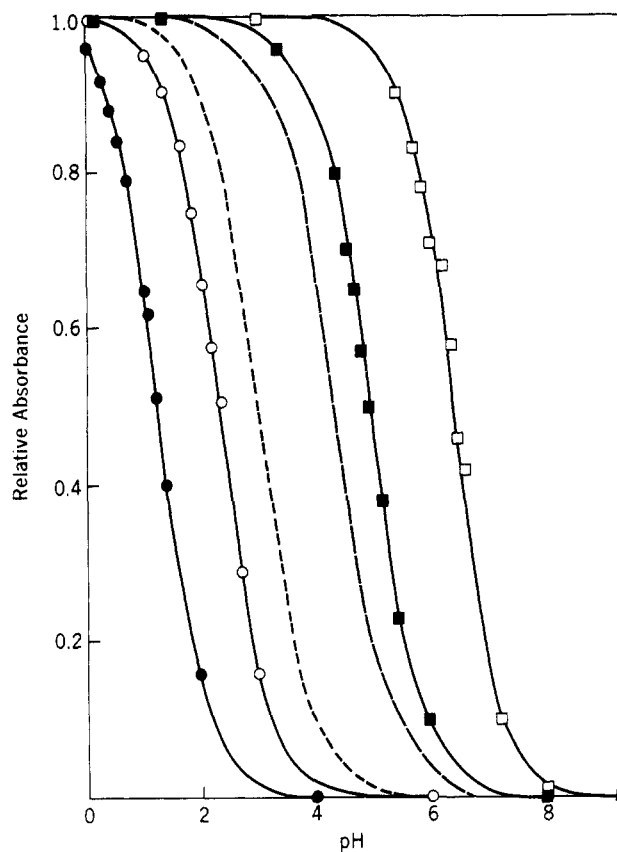
Scheme I

Figure 1. Relative flavylium ion absorbance (absorbance/absorbance in acid) as a function of pH: (□) 3-methoxy-5,6-dihydrobenzo[c]xanthylium, λ = 452 nm; (■) 5,6-dihydrobenzo[c]xanthylium, λ = 420 nm; (○) 4′-methoxy-3-methylflavylium, λ = 430 nm; (·) 3-methylflavylium, λ = 387 nm. Solutions were (2–5) × 10^{-5} M in cation; the concentration within each series was held constant. Absorbance readings were obtained 10–60 s after solution preparation. The acid absorbance reading for 3-methylflavylium is based on extrapolation. The dashed lines are theoretical curves[1] for the 4′-methoxyflavylium ion (– –) and the flavylium on (- - -).

summarize these experiments and their significance. The discussion will revolve around Scheme I which comes from the previous study and shows species involved and rate and equilibrium constants relating these. Water addition and, at higher pH, hydroxide ion addition occur reversibly at position C2 of the flavylium ion (C12a of the benzo[c]-xanthylium cation) to produce the pseudobase[8,9] B2. This product is a cyclic hemiacetal which opens reversibly to the chalcone ZC in a reaction catalyzed by acids, bases, and water (pH independent process), as is typical of hemiacetal decomposition reactions.[10] The interconversions of F$^+$, B2, and ZC are all relatively fast. Where the (Z)-chalcone does exist in appreciable quantities in the overall equilibrium, there occurs a slow irreversible isomerization to the (E)-chalcone EC.

Apparent Acidity Constants. A stock acetonitrile solution of the flavylium ions was prepared and added to aqueous solutions of pH 0–9. As shown in Figure 1 the cation equilibrates relatively rapidly with colorless forms in a typical acid–base reaction.

$$\text{F}^+ \rightleftharpoons \text{neutral} + \text{H}^+ \qquad (1)$$

Acidity constants were calculated (see Table I for values of all constants) by using the relationship

$$K_{obsd}{}^a(\text{F}^+) = \frac{A_{acid} - A}{A - A_{neut}}[\text{H}^+] \qquad (2)$$

where A_{acid} and A_{neut} refer to acid and neutral solutions ($A_{neut} \approx 0$ at the λ_{max} of the cation) and A is the absorbance at intermediate pH.

For the 3-methylflavylium ion a value of A_{acid} could not be obtained since even in 1.0 M HCl the cation absorbance has not reached a level value. In this case the absorbance
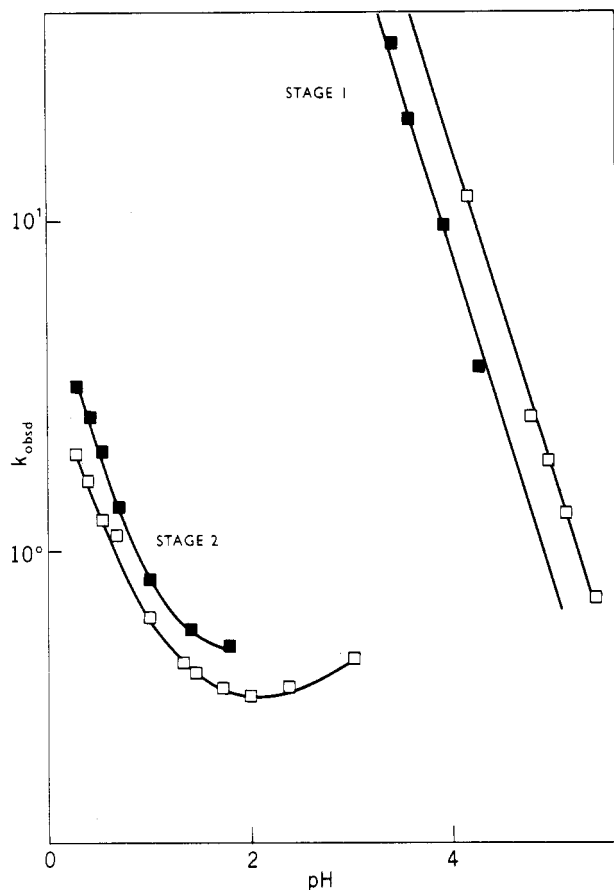
(8) Bunting, J. W. *Adv. Heterocycl. Chem.* **1979**, *25*, 1.

(9) In the previous study there was evidence for a pseudobase B4 derived from addition at C4 of the flavylium ion. This pseudobase is a kinetic product only, rapidly converting to the more stable B2 form on standing. There is evidence for the B4 pseudobase with the flavylium ions of this study, but it has been omitted in this presentation in order to keep the discussion simple. It can also be noted that with a hydroxy substituent at appropriate positions (C4′, C5, C7) an anhydro base derived by its deprotonation is also possible.

(10) (a) Funderburk, L. H.; Aldwin, L.; Jencks, W. P. *J. Am. Chem. Soc.* **1978**, *100*, 5444. (b) McClelland, R. A.; Coe, M. *J. Am. Chem. Soc.* **1983**, *105*, 2719.

Figure 2. First-order rate constants for formation of 5,6-di-hydrobenzo[c]xanthylium ions on acidification of pH 8 solutions: (□) 3-methoxy; (■) unsubstituted. The rate constants for stage 1 continue to increase with increasing acidity, and below pH 2 this process is too fast to be observed using the stopped-flow technique. Stage 2 is observed with both systems at pH 3-5, but rate constants are very dependent upon buffer concentration.[11]

data were fit to a rearranged form of eq 2 using nonlinear least squares to provide both $K_{obsd}{}^a(F^+)$ and A_{acid}.

Reacidification. In these experiments the flavylium ion is placed in a solution of sufficiently high pH that it is completely decolorized. This solution is then mixed with various acid solutions and the reformation of the colored cation observed by using stopped-flow spectroscopy. Three different types of behavior were observed.

(a) The two dihydrobenzo[c]xanthylium ions reform in two quite distinct stages. This behavior was found with all three cations in our previous study[1] (see Figure 9 of ref 1). The first phase consists of a rapid increase in absorbance, the absorbance starting near zero and rising to some intermediate value. This change is first order, and with varying pH the first-order rate constants are found to be proportional to hydronium ion concentration (Figure 2). The second phase is considerably slower, the absorbance undergoing a further increase to a stable final value corresponding to quantitative regeneration of cation. This stage also exhibits first-order kinetics; the pH dependency of the rate constants is more complex, with H^+-dependent, OH^--dependent, and pH-independent regions being present (Figure 2).

This biphasic behavior can be explained by having the neutral solution before mixing consist of a mixture of the pseudobase B2 and the chalcone ZC. On acidification the pseudobase is rapidly converted to cation in a H^+-catalyzed reaction.

$$k_{obsd}(\text{stage 1}) = k_{-1}{}^H[H^+] \quad (3)$$

This results in a solution which still contains chalcone, and

the second phase represents conversion of this species to cation. This necessarily occurs via the pseudobase, and the cyclization ZC → B2 is rate-limiting, since in acids B2 → F^+ is very fast. Thus

$$k_{obsd}(\text{stage 2}) = k_{-2}{}^H[H^+] + k_{-2}{}^W + k_{-2}{}^{OH}[OH^-] \quad (4)$$

Values of the various rate constants obtained for both stages are given in Table I. The very rapid base-catalyzed interconversion of chalcone and pseudobase ($k_{-2}{}^{OH} \approx 10^{10}-10^{11}$ M^{-1} s^{-1}) has been the subject of a detailed study[11] and will not be discussed here.

A further parameter is available from this experiment, since the total changes in absorbance which occur in the two stages are proportional to the concentrations of pseudobase and chalcone before acidification. Since the pseudobase and chalcone are in equilibrium before mixing[1]

$$\frac{\Delta OD(\text{slow})}{\Delta OD(\text{fast})} = \frac{[ZC]}{[B2]} = K_2 \quad (5)$$

Knowledge of K_2 then leads to other constants of Scheme I. The constants $k_2{}^H$, $k_2{}^W$, and $k_2{}^{OH}$ are determined from the relationship $K_2 = k_2{}^{cat.}/k_{-2}{}^{cat.}$, where "cat" refers to any catalysis. The equilibrium constant $K_1{}^a$ is calculated from

$$K_{obsd}{}^a(F^+) = K_1{}^a(1 + K_2) \quad (6)$$

this equation being derived on consideration that the "neutral" species in equilibrium with cation in eq 1 is a mixture of pseudobase and chalcone. Knowledge of both $K_1{}^a$ and $k_{-1}{}^H$ then provides $k_1{}^W$.

(b) The 4'-methoxy-3-methylflavylium ion also forms in two stages but they are poorly separated (Figure 3), and it is not possible to measure individual first-order rate constants or to determine the value of K_2 from relative absorbance changes. What is happening here is that
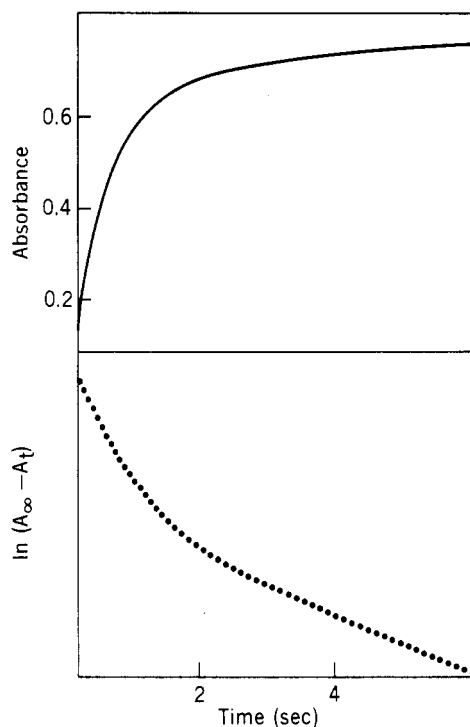

Figure 3. Formation of the 4'-methoxy-3-methylflavylium ion in 0.05 M HCl on acidification of pH 7 solution. The top curve plots the absorbance at 430 nm vs. time. The bottom curve is the result of a first-order plot, where A_∞ is the final stable absorbance reading.

(11) McClelland, R. A.; Devine, D. B.; Sørensen, P. E. *J. Am. Chem. Soc.* **1985**, *107*, 5459–5463.

Table II. Rate Constants for the Formation of the 4'-Methoxy-3-methylflavylium Ion

pH	k_1	k_{-1}	$k_1[H^+]/k_{-1}$	k_2	k_{-2}	k_2/k_{-2}
1.82	3.08	11.48	0.0041	0.49	2.40	0.20
1.70	3.04	14.7	0.0041	0.58	2.80	0.20
1.60	2.93	18.11	0.0041	0.64	3.37	0.19
1.52	3.73	25.69	0.0044	0.86	4.04	0.21
1.40	3.28	29.60	0.0044	1.04	5.19	0.20

pseudobase and (Z)-chalcone are present in the neutral solution as in the previous example but now the rate for B2 → F$^+$ is not that much faster than the rate for ZC → B2. This system is also complicated by the fact that with pK_{obsd}^a (F$^+$) being only equal to 2.28, flavylium ion is not quantitatively regenerated except in the most concentrated acids. The kinetic system then takes the form

$$ZC \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} B2 \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} F^+ \qquad (7)$$
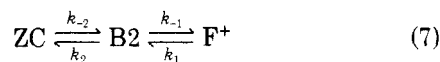
where k_{-2}, k_2, k$_{-1}$, and k$_1$ are the first-order rate constants at a particular pH. An expression can be derived for the F$^+$ concentration as a function of time, and by inputting one parameter, the fraction of flavylium which has formed upon completion, it is possible to fit the experimental curves to obtain the individual constants (see Appendix). These are listed in Table II, and the constants do take the form expected. The constant $k_{-1} = k_{-1}^H[H^+]$ since it refers to H$^+$-catalyzed conversion of the pseudobase to cation. The constant k_1 represents the microscopic reverse, water addition to cation (k_1^W) and is pH independent. The quantity $k_1/k_{-1}[H^+]$ is constant; this is the equilibrium constant K_1^a. The constants k_2 and k_{-2} obey the relationship $k_i = k_i^W + k_1^H[H^+]$, where $i = 2$ or -2. Their ratio k_2/k_{-2} is constant, since this is simply the equilibrium constant K_2.

(c) Acidification of the neutral solution of the 3-methylflavylium ion results in a single stage kinetic trace exhibiting reasonably good first-order kinetics. If one considers the difference in K_2 between 4'-H- and 4'-MeO-substituted cations (see Table I), then the value of K_2 in the 3-methyl system can be estimated to be of the order of 0.02–0.03. This means that there is only a very small amount of (Z)-chalcone in the neutral solution, and its presence is not detected in the reacidification experiment. The observed first-order curves therefore represent formation of flavylium ion from pseudobase. Since an equilibrium mixture of the two is the result even in concentrated HCl, the observed rate constants represent forward and reverse rate constants.

$$k_{obsd} = k_{-1}^H[H^+] + k_1^W \qquad (8)$$

A plot of k_{obsd} vs. [H$^+$] has a slope (k_{-1}^H) of 3.0×10^2 M^{-1} s^{-1} and an intercept (k_1^W) of 20. The ratio of these two expressed as log k_{-1}^H/k_1^W is equal to pK_1^a which is approximately pK_{obsd}^a(F$^+$) since K_2 is low. The numerical value obtained from the kinetics is 1.18, which is in good agreement with the value of 1.22 obtained spectroscopically.

Chalcone Isomerization. In solutions with pH > pK_{obsd}^a(F$^+$) a slow further reaction occurs with all four flavylium ions, resulting in quantitative formation of a product which does not reform cation upon reacidification. This can be identified as the isomerized (E)-chalcone. This was verified in one case by preparing such a species by base-catalyzed aldol condensation, and showing it to be spectroscopically identical with that obtained from the flavylium ion. Rate constants were measured in 0.01 M NaOH for the chalcone from the 3-methylflavylium ion and from the 5,6-dihydrobenzo[c]zanthylium cation. These are listed[12] as k_3' in Table I. The rates in neutral (pH 7)

solutions were considerably slower and were not measured.

Discussion

Of the various transformations identified and studied in this report, the one which is most dramatically changed by the substitution is the equilibrium hydration of the cation itself. 3-Methyl substitution causes a decrease in pK_1^a by a factor of about 2, so that in consequence more acidic solutions are required to maintain the 3-methylflavylium ions in their colored forms. Interestingly the introduction of the bridging ethylene unit results in almost exactly the opposite effect, with pK_1^a values about two log units greater than those for the 3-H-substituted cations (and four units greater than the 3-methyl systems). The methoxy-substituted bridged cation in consequence is stable to quite high pH. On electronic grounds the 3-alkyl-substituted cations would have been predicted to be more stable than their unsubstituted analogues, so that the decreased stability observed with the 3-methyl system must be steric in origin. One explanation involves a steric interaction of the 3-substituent and an ortho (2') hydrogen of the phenyl ring. Such an interaction could force the
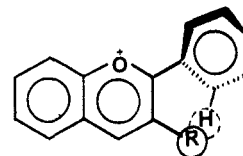
phenyl ring to move out of coplanarity with the benzopyrylium portion of the molecule, destabilizing the cation because of decreased delocalization of the positive charge. The ethylene-bridged cations provide the model for a planar system. The decreased stability of the parent ions (3-H substituent) is explained by some distortion due to this interaction even in these cations. 3-Methyl substitution results in further twisting and concomitant decrease in stability. Interestingly a crystal structure of a 4',6,7-trihydroxyflavylium salt (3-H-substituted) reveals a phenyl ring 5.9° out of planarity with the benzopyrylium,[13] and this increases to 10.1° on 3-OH substitution, in a 3,5,7,3',4'-pentahydroxyflavylium (cyanidin) salt.[14]

There are additional pieces of evidence supporting the above interpretation. With each cation a para-methoxy substituent in the phenyl ring stabilizes the cation, as expected, but if the degree of stabilization is considered (as pK_1^a(p-MeO) – pK_1^a(H)), then the effect is seen to be greatest with the two benzoxanthylium ions and least with the two 3-methylflavylium ions. This implies that the conjugative interaction of the methoxy substituent and the benzopyrylium ion is greatest for the bridged ion and least for the 3-methyl ion. This is, of course, consistent with the idea that there are varying degrees of coplanarity in

(12) In our previous study we identified two isomerization processes, one (k_3') occurring in concentrated base involving isomerization of the (Z)-chalcone anion and one (k_3) occurring at neutral pH involving isomerization of the neutral chalcone.

(13) Ueno, K.; Saito, N. *Acta Crystallogr., Sect. B.: Struct. Crystallogr. Cryst. Chem.* **1977**, *B33*, 111.

(14) Ueno, K.; Saito, N. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1977**, *B33*, 114.

the three cations. There is in addition a significant shift in the λ_{max} of the cation, increased conjugation of the aryl ring and the benzopyrylium ion leading to a bathochromic shift. This observation is important since the parameter being measured here is a property of the cation only (and its excited state).

Acknowledgment. The continued financial support of the Natural Engineering and Research Council of Canada is gratefully acknowledged.

Appendix. Kinetic Analysis. The Formation of the 4'-Methoxy-3-methylflavylium Ion from Neutral Solutions

The kinetic system is defined as

$$ZC \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} B2 \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} F^+ \qquad (A1)$$
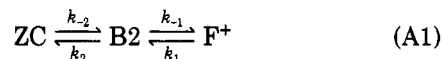
and the boundary conditions that at zero time, $[F^+] = 0$ and $[ZC]/[B2] = k_2/k_{-2}$, the following expression can be derived[15] for the flavylium ion absorbance

$$\frac{A_\infty - A}{A_\infty} = C_1 e^{-C_2 t} + (1 - C_1) e^{-C_3 t} \qquad (A2)$$

(15) Bernasconi, C. F. "Relaxation Kinetics"; Academic Press: New York, 1976.

where A_∞ is the final absorbance value in the solution in question and

$$C_1 = \frac{\dfrac{k_1 k_2 + k_1 k_{-2} + k_{-1} k_{-2}}{k_2 + k_{-2}} - C_2}{C_3 - C_2} \qquad (A3)$$

$$C_2, C_3 = \tfrac{1}{2}\{(k_1 + k_{-1} + k_2 + k_{-2}) \\ \pm \sqrt{(k_1 + k_{-1} + k_2 + k_{-2})^2 - 4(k_1 k_2 + k_1 k_{-2} + k_{-1} k_{-2})}\}$$

The experimental absorbance:time curves were fit by using nonlinear least squares to provide values of C_1, C_2, and C_3 at each pH. These provide three equations in the constants k_1, k_2, k_{-1}, and k_{-2}.

$$\frac{k_1 k_2 + k_1 k_{-2} + k_{-1} k_{-2}}{k_2 + k_{-2}} = C_1 (C_3 - C_2) + C_2 \qquad (A5)$$

$$k_1 + k_{-1} + k_2 + k_{-2} = C_2 + C_3 \qquad (A6)$$

$$k_1 k_2 + k_1 k_{-2} + k_{-1} k_{-2} = C_2 C_3 \qquad (A7)$$

A fourth equation is available using the observed acidity constant

$$\frac{k_{-1} k_{-2}}{k_1 k_2 + k_1 k_{-2} + k_{-1} k_{-2}} = \frac{[H^+]}{[H^+] + K_{obsd}{}^a (F^+)} \qquad (A8)$$

Algebraic manipulation of these four equations provides the four individual constants.

Metalation of Isoxazolyloxazolines, a Facile Route to Functionally Complex Isoxazoles: Utility, Scope, and Comparison to Dianion Methodology

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2-(5'-Alkylisoxazol-4'-yl)-Δ^2-oxazoline was metalated at the C-5' alkyl group, and the lithio anion was quenched with a variety of electrophiles. Alkyl halides, aldehydes, and acylpyridinium salts were used as electrophiles. The lithio anion was oxygenated with MOOPH or *N*-(phenylsulfonyl)oxaziridene. The isoxazolyloxazoline system was converted to the isoxazolyl carboxylic acid, aldehyde, ketone, and chiral oxazoline. The isoxazolyloxazoline was formed, metalated, and deprotected in synthetically useful yields and represents a facile entry into functionally complex isoxazoles. To determine the necessity of the oxazoline protection/deprotection scheme, dianions of isoxazole-4-carboxylic acids were studied. The dianion method was found to be more efficient for simple alkyl halides, but limited in scope.

Isoxazoles continue to be of interest both for their synthetic utility and intrinsic biological activity.[1]   In the course of related studies[2] we desired a facile entry into derivatives containing a carbonyl functional equivalent in the C-4 position of the isoxazole ring. We have examined the use of the oxazoline protecting group[3] for the carboxyl

group, and herein report in full on the utility, scope, and limitations of this approach.[4] We have also examined the formation and utility of dianions of isoxazole-4-carboxylic acids to weigh the relative merit of the use of the oxazoline protecting group.

The metalation of simple alkylisoxazoles, termed "lateral" metalation by Micetich,[5] is a useful synthetic

(1) (a) For a review, see: Wakefield, B. J.; Wright, D. J. *Adv. Heterocycl. Chem.* 1979, *25*, 147. (b) For a recent example of isoxazoles with useful biological activity see: Diana, G. D.; McKinlay, M. A.; Brisson, C. J.; Zalay, E. S.; Miralles, J. V.; Salvador, U. J. *J. Med. Chem.* 1985, *28*, 748.
(2) (a) Natale, N. R. *Tetrahedron Lett.* 1982, 5009. (b) Natale, N. R.; Quincy, D. A. *Synth. Commun.* 1983, *13*, 817.

(3) (a) For a recent review on oxazoline chemistry see: Reuman, M.; Meyers, A. I. *Tetrahedron* 1985, *41*, 837. (b) Meyers, A. I.; Mihelich, E. D. *Angew. Chem., Int. Ed. Engl.* 1976, *15*, 270.
(4) Part of this work has been communicated in preliminary form: Natale, N. R.; Niou, C. S. *Tetrahedron Lett.* 1984, *25*, 3943.
(5) Micetich, R. G. *Can. J. Chem.* 1970, *48*, 2006.