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Alkaline hydrolytic lability of some hydroxy- and methoxycoumarins and related anticoagulants

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The susceptibility of coumarins to alkaline hydrolysis, resulting in the formation of cis-coumarinate (and subsequently trans-coumarinate) polyanions is well known (Decker and Becker, 1922; Garrett et al., 1971; Mattoo, 1957; Bowden et al., 1968; Lippold and Garrett, 1971). For the most part, studies of substituent effects on the rates of alkaline hydrolysis of coumarins have been limited to derivatives with relatively weakly interacting functional groups. However, the coumarins of greatest economic interest are hydroxylated derivatives some of which are used as anticoagulants, fluorescent probes and optical components of mode-locked lasers (Shank et al., 1970; Trozzolo et al., 1974; Schulman and Rosenberg, 1979). The resistances of these substances to decomposition (Connors et al., 1979) under various solution conditions is therefore, of more than academic interest.

The ionization of the hydroxy group which ought to play a dramatic role in the relative labilities of variously substituted hydroxycoumarins to attack by OH^-, introduces an excellent opportunity to assess the relative importances of strong conjugative and electrostatic influences on the hydrolysis of coumarins.

In the present note we consider the influence of the position of the hydroxy group on the second-order rate constants for the hydrolysis of 3-, 4- and 7-hydroxycoumarin and some related anticoagulants—warfarin, acenocoumarol and phenprocoumon—the drugs of choice in the U.S.A., The Netherlands and the Federal Republic of Germany, respectively. Because the hydroxy groups of all the compounds studied are fully ionized at the pH where the hydrolyses were studied

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[1] The value of $pK_a = 7.12 \pm 0.05$ at 22°C was recently reported for the 3-isomer (Wolfbeis, 1981).

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(the pK_a values at 25°C, determined spectrophotometrically, in the present work and extrapolated to infinite dilution, were 6.87 [1] ± 0.02, 4.12 ± 0.03 and 7.88 ± 0.05 for 3-, 4- and 7-hydroxycoumarin, respectively), the methyl ethers of the simple hydroxycoumarins were prepared and their hydrolyses studied as well. The methoxy derivatives are taken to be approximations of the undissociated hydroxy compounds. As well as being chemically interesting themselves, their rates of hydrolysis allow rapid estimates of how much hydrolysis of the neutral species is contemporaneous with hydrolysis of the anions at a given pH. All compounds were obtained from commercial sources except for 3-hydroxycoumarin (Sen and Baghi, 1959, 1960) and the methyl ethers of 4-hydroxycoumarin, 7-hydroxy-4-methylcoumarin (Yakatan et al., 1972a and b) and 3-hydroxycoumarin (Hulshoff et al., 1979) whose preparations were taken from the literature. Laser grade 7-diethylamino-4-methylcoumarin was a gift from Professor Howard Latz, Department of Chemistry, University of Ohio, Athens, OH.

Approximately 5×10^{-3} M solutions of the coumarin derivatives were prepared in 95% ethanol. Between 10 and 30 μl of each solution were pipetted into 2 ml of each of several standard NaOH solutions using a variable volume Finnpipet. The NaOH solution concentrations varied from 10^{-3} to 10^{-1} M in order to effect complete hydrolysis in between 45 min and 4 h. The ionic strength was adjusted to 0.1 if necessary with KCl. Electronic absorption spectra were repetitively scanned at 2–10 min intervals using a Cary 219 spectrophotometer with a sample compartment whose temperature was controlled, by means of a water bath, at 25 ± 0.5°C. Three different analytical wavelengths were employed and straight line plots of $\ln(A-A_\infty)$, where A_∞ is the absorbance at complete hydrolysis, vs time, were constructed using the first 10 data points (scans). A calculator routine stored the A, t data points and constructed linear plots by testing different values of A_∞, storing the A_∞ which gave the highest correlation coefficient. This procedure was used because a loss of

TABLE I

SECOND-ORDER RATE CONSTANTS (k) FOR THE ALKALINE HYDROLYSIS OF SEVERAL HYDROXY- AND METHOXYCOUMARINS AT 25°C AND IONIC STRENGTH $\mu = 0.10$

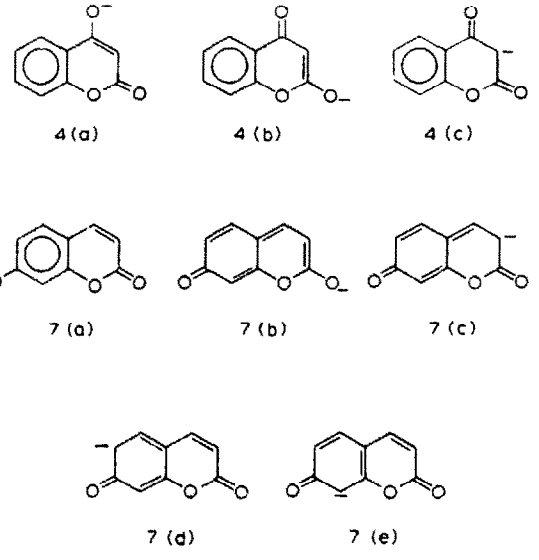
Compound	k ($M^{-1} S^{-1}$)
Coumarin	$4.03 \pm 0.03 \times 10^{-1}$
3-Hydroxycoumarin (anion)	$1.00 \pm 0.01 \times 10^{-1}$
4-Hydroxycoumarin (anion)	very slow ($k < 10^{-7}$)
Warfarin (anion)	very slow ($k < 10^{-7}$)
Acenocoumarol (anion)	very slow ($k < 10^{-7}$)
Phenprocoumon (anion)	very slow ($k < 10^{-7}$)
7-Hydroxycoumarin (anion)	$7.67 \pm 0.02 \times 10^{-3}$
7-Hydroxy-4-methylcoumarin (anion)	$6.26 \pm 0.03 \times 10^{-3}$
3-Methoxycoumarin	2.13 ± 0.02
4-Methoxycoumarin	$6.58 \pm 0.02 \times 10^{-3}$
7-Methoxy-4-methylcoumarin	$4.52 \pm 0.02 \times 10^{-2}$
7-Diethylamino-4-methylcoumarin	$7.07 \pm 0.01 \times 10^{-3}$

isosbestic points was often observed after a few hours. This signalled the occurrence of a secondary, slower reaction; presumably the geometrical isomerization of the cis-coumarinate anions formed by hydrolysis.

The second-order rate constants (k) for the alkaline hydrolyses of the coumarins studied are given in Table 1. From the data of Table 1 several observations can be made. (a) At pH > 11, the products of the second-order rate constant, k, and the dissociation constant, K_a, for the hydroxycoumarins, are at least 20 times greater than the product of k for the methoxycoumarins and $[H^+]$. This indicates that at pH > 11, the hydroxycoumarins are hydrolyzed virtually exclusively as the anions. (b) All of the anionic hydroxycoumarins are hydrolyzed considerably slower than is coumarin. (c) The methoxycoumarins which were available hydrolyze more rapidly than the corresponding hydroxycoumarin anions. (d) The rate constants for the hydrolyses of 7-substituted, 4-methylcoumarins are in the order 7-methoxy > diethylamino > hydroxy(anion), the opposite of the order of lone pair delocalizability of the 3 functional groups in the 7-position, (e) For both the dissociated hydroxy- and methoxy series 4-substitution is more effective at protecting the lactone ring from hydrolysis than 7-substitution which, in turn, is more effective than 3-substitution. These observations lead to the conclusion that the electronic charge placed at the carbonyl group of the lactone ring by the various hydroxy and methoxy substituents inhibits attack on this group by nucleophiles such as OH^-, slowing the formation of the tetrahedral intermediates which are precursors to the final hydrolysis products.

The relative electron-donating abilities of the various groups affect the rates of hydrolysis accordingly.

That 3-methoxycoumarin is hydrolyzed appreciably faster than coumarin is in



Scheme 1.

line with the ability of the 3-methoxy group to influence the rate of hydrolysis only by electron-withdrawing inductive and field effects. Only one stable mesomeric form can be drawn for the 3-methoxy derivative. Similarly, the fact that only one stable mesomer can be drawn for the dissociated 3-hydroxycoumarin explains the relatively weak effect the ionized 3-hydroxy group has on slowing the rate of hydrolysis.

In the cases of the 4-hydroxy- and 7-hydroxycoumarins, by analogy with the 3-hydroxy and methoxy compounds, the distance dependences of the field and inductive effects would predict that the dissociated 4-hydroxyanion should hydrolyze more slowly than the 7-hydroxycoumarin and this is observed (but on a much greater scale than expected—the 4 isomer appears to be quite inert to hydrolysis). But the electron-withdrawing influence of the methoxy group seen in 3-methoxycoumarin, is not obvious in the 4- and 7-methoxy compounds as their rates of hydrolysis are slower than those of coumarin. The 4-isomer which should show greater field and inductive effects than the 7-methoxy derivative actually hydrolyzes at a slower rate. Obviously, resonance effects are dominant in the 4- and 7-substituted coumarins. For the 4-hydroxycoumarin anion three, and 7-hydroxycoumarin anion five, more or less stable valence-bond structures can be drawn (Scheme 1). In 7-hydroxycoumarin, mesomers 7 (b), (d) and (e) are somewhat destabilized by the necessity to disrupt an aromatic sextet in the homocyclic ring in order to form them and mesomer 7(c) is only weakly stabilized by the presence of a negative charge on the carbon atom α to the single carbonyl group in the molecule. However, the ability to delocalize negative charge into the aromatic ring in the 7-isomer may leave the 2-carbonyl group more susceptible to nucleophilic attack. In contrast, none of the mesomeric forms of the 4-isomer require disruption of an aromatic sextet and the carbanion structure in 4 (c) is well stabilized by virtue of its location α to two carbonyl groups. The greater resonance stabilization of the negative charge at or near the 2-carbonyl group of the anion of the 4-hydroxycoumarin accounts for its greater hydrolytic inertness as well as the lower pK_a of its conjugate acid, relative to those properties of the 7-isomer.

It has been pointed out that 'thorough kinetic studies of warfarin (and 4-hydroxycoumarin) do not appear to have been reported' (Connors et al., 1979). This can now be stated to be the result of the extreme lack of hydrolytic reactivity of anionic 4-hydroxycoumarin and its pharmaceutically significant derivatives warfarin, acenocoumarol and phenprocoumon. In the present study it was found that even after 6 months in 2 M NaOH, these 4-hydroxycoumarin derivatives failed to demonstrate any apparent reaction.

A rather interesting peripheral aspect of the stabilities of the 4-hydroxycoumarins in alkaline solutions is that the pH-tunable dye lasers, many of which are derived from coumarins such as 7-hydroxycoumarin and 7-diethylaminocoumarin, could conceivably be stabilized for operation under alkaline conditions by the introduction of a hydroxy group in the 4-position. Such compounds have been synthesized (Wolfbeis, 1977; Wolfbeis and Lippert, 1978) and ought to be investigated more thoroughly.

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