SPECTROPHOTOMETRIC STUDY OF THE HYDROLYSIS OF COUMARIN AND DISSOCIATION OF *CIS*-COUMARINIC ACID

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The hydrolysis of the pH-labile coumarin ring in the dark and the subsequent dissociation of *cis*-coumarinic acid involve three equilibria. The first equilibrium, coumarin HOH

 $\overrightarrow{}$ cis-coumarinic acid ion (HA⁻) + H⁺ is attained slowly; pK_h (therm.) at 25° C = 9.56. The second, HA⁻ \rightleftharpoons A²⁻ + H⁺, has pK₂ (therm.) at 25° C = 10.66. The third equilibrium may involve the interaction of HA⁻ and A²⁻, and it is pH-dependent.

The effect of irradiation of *cis*-coumarinic acid ion (A^{2-}) has been studied. A borate complex with *cis*-coumarinic acid ion (HA^{-}) is reported.

In continuation of the author's work ¹ on the absorption and fluorescence spectra of hydroxy-coumarins, it was desirable to study their dissociation constants by spectrophotometric or spectrofluorimetric methods. Potentiometric and conductometric methods are unsuitable because of the decomposition of the coumarin ring in alkali; in any case the measurements would have been less precise because of the low solubility of these compounds in water. Prior to the investigation of the hydroxy derivatives it was necessary to study the behaviour of the unsubstituted coumarin ring at alkaline pH. The results of this study are presented in this paper.

Coumarin hydrolyzes to non-fluorescent *cis*-coumarinic acid in the dark at alkaline pH and the equilibrium is attained in *ca*. 12 h. On exposure to light a strong green fluorescence is developed which may be attributed to photolysis and *cis-trans*-isomerization (see later). Due care was taken to avoid these difficulties in the study of hydrolysis and subsequent dissociation.

EXPERIMENTAL

The absorption measurements were done with a Beckman DU spectrophotometer using matched silica cells (0.996 cm). The cells were thermostatted at 25° C. Water from a bath maintained at $25 \pm 0.1^{\circ}$ C was circulated through two jackets enclosed in the cell compartment mounting block. The temperature was attained fairly rapidly. There was, however, slight heating of *ca*. 0.3 deg. by the hydrogen lamp. Tap water $< 25^{\circ}$ C) was circulated through the lamp housing. Aqueous coumarin solutions at concentration = $1.23_7 \times 10^{-4}$ M were studied; the coumarin sample was recrystallized and its m.p. agreed with the literature value. Beer's law was found valid in this concentration range. The pH was adjusted with Na₂HPO₄ and NaOH with progressive dilution. Complex formation of *cis*-coumarinic acid with borate (see later) rendered boric acid+NaOH buffers useless. The pH was measured at 25° C with C.I. Co. glass electrodes (Alki electrode at pH > 9), using a Pye potentiometer supplemented with electrometer valve (ET₁) amplification. Standardization of the electrode assembly was done with Muirhead and Coleman buffer tablets.

The solutions were mixed and preserved in the dark. Coumarin solutions in Jena-glass stoppered bottles covered with photographic dark paper were mixed with buffers under red light. The absorption cells were filled under red light and the cell holder was covered with dark paper before putting it in position in the spectrophotometer. It was observed that whatever irradiation the solutions were exposed to in the spectrophotometer, it was

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too feeble to bring about significant changes in the optical density; neither was there any detectable fluorescence developed by the time the absorption measurements were over. These solutions, practically non-fluorescent, developed strong green fluorescence on exposure to a 125 W Wood's lamp. Before mapping out the whole spectrum, absorption was measured at 3 wavelengths of interest, viz., 240, 280 and 350 m μ , and these values were used in subsequent calculations. These considerations explain why the incidental data on absorption of coumarin at different pH given by Harle and Lyons,² and Patzak and Neugebauer ³ (supplementing their polarographic study) are in error and at variance with each other. The data in the former paper are obviously erroneous in the range 350-360 m μ and that in the latter in the range 260-300 m μ . Both fail to obtain the isobestic point at 263 m μ . The time required to attain the hydrolytic equilibrium reported by Patzak (*ca.* 48 h) is also high. The data in fig. 1 show that in ~ 12 h the equilibrium is practically reached. In this study the solutions were kept for equilibration for 24-48 h.



110. 1.

RESULTS

Assuming the equilibria



and

$$\begin{array}{c} OH \\ COO^{-} \\ \end{array} \rightleftharpoons \begin{array}{c} O^{-} \\ COO^{-} \\ + H^{+} \end{array}, \text{ where } = \frac{c_{3}f_{3}a}{c_{2}f_{2}}.$$
 (2)

it can be shown that 4

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$$D_n K_h - D_2 K_h + \left[(D_n - D_3) \left(f_2 / f_3 \right) / a_n \right] K_h K_2 = (D_1 - D_n) a_n f_2.$$
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(3)

Here ϵ , c, f refer to molar extinction coefficients, molar concentrations and mean ionic activity coefficients of the particular species, respectively. K_h refers to the hydrolysis constant of coumarin and K_2 to the second dissociation constant of *cis*-coumarinic acid; while a denotes H⁺ activity. The subscript n stands for the particular nth solution whose optical density corrected for buffer and solvent is D_n . D_3 refers to the optical density at the same concentration in strongly alkaline medium, while D_1 to that in an acid solution. To solve eqn. (3) for the unknowns K_h , K_2 and D_2 , the minimum data required are those at 3 different pH.

A number of such sets of equations were framed from absorption data at more than 3 different pH and more than one wavelength. However, negative values for one of the unknowns were consistently obtained showing thereby the inadequacy of eqn. (3). The only possibility was the presence of some other equilibrium which was not considered in deriving this relation. It was confirmed that the acidic curve and the highly alkaline curve did faithfully represent the absorption of coumarin molecule and *cis*-coumarinic acid anion respectively, and that there was no interaction between the constituents in either of these.

The equilibrium



cannot exist because hydrolysis occurs at too high a pH to allow any *cis*-coumarinic acid to exist in the molecular form, H₂A. The *trans*-isomer of this acid, which exists in the free state, has a $pK_1 = 4.52$ ⁵ (4.61).⁶ The *cis*-isomer from theoretical considerations, will have a still lower pK_1 (see later). Thus even at pH 7, where there is practically no hydrolysis, H₂A will almost be completely dissociated to HA⁻.

It was also found that the additional equilibrium could not be one of a tautomeric nature. Assuming the equilibrium



in addition to (1) and (2) it can be shown that

$$D_n K_h - D_2 K_h + [(D_n - D_3)(f_2/f_3)/a_n] K_2 K_h + D_n K_t K_h - D_4 K_t K_h = (D_1 - D_n) a_n f_2.$$
(4)

This equation involves 5 unknowns, viz. K_h , K_t , K_2 , D_2 and D_4 . On setting up five such equations these were again found to yield no results, ruling out, thereby, the possibility of such an equilibrium.

The other possibility could be that of an interaction between the singly and doubly charged *cis*-coumarinic acid anions :



where $K'_d = (c_4 f_4 a_0 \bar{\mathbf{n}})/(c_2 c_3 f_2 f_3)$. This being a pH-dependent equilibrium, it is possible to establish its presence qualitatively from consideration of the isobestic points. With additional absorption data at different pH it was possible to divide these curves into three families passing through the isobestic points at 335, 316 and 308.5 m μ respectively (fig. 2). The isobestic point at 335 m μ can then be interpreted in terms of the equilibrium (1), where $\epsilon_1 = \epsilon_2$. The isobestic point at 308.5 m μ is due to $\epsilon_2 = \epsilon_3$ (equilibrium (2)). Without going into a detailed treatment of the equilibria (1), (2) and (5), which involves laborious calculations (see appendix), it is possible to study the hydrolysis independently



in the pH range < 9.3 and the second dissociation of *cis*-coumarinic acid independently in the pH range > 10.3.



From (1) it can be shown that for the family of curves passing through the isobestic point at 335 m μ ,

$$D_n K_h - D_2 K_h = (D_1 - D_n) a_n f_2.$$
(6)

Eqn. (6) can be solved for K_h . Absorption data for the most reliable set of pHs, viz. HCl, 8.85, 9.06 from which pK_h has been calculated, are presented below.

•	optical			
λ (mμ)	$\sim 0.08 \text{ N}$ HCl	pH = 8.85 $I = 0.0029$	pH = 9.06 $I = 0.003_6$	pK _h (25°)
240	0.215	0.323	0.373	9.52
280	1.327	1.182	1.113	9.57
310	0.767	0.700	0.668	9.58
			m	ean = 9.56

Activity coefficients were estimated by Davies' equation : 7

$$-\log f = 0.5 Z^2 \left[\sqrt{I} / (1 + \sqrt{I}) - 0.2 I \right].$$

At very low I, this approximates to the Debye-Hückel limiting formula.

It can similarly be shown from (2) that for the family of curves passing through the isobestic point $308.5 \text{ m}\mu$,

$$[(D_n - D_3)(f_2/f_3)/a_n]K_2 - D_2 = -D_n.$$
⁽⁷⁾

	This equation	can be solve	d for K_2 . A	bsorptior	n data for t	he most i	reliable set	of pHs
iz.	KOH, 10·62,	10·34, from	which pK_2 h	as been o	calculated a	are prese	nted below.	

1	optical			
(mμ)	~0.5 N KOH	pH = 10.62 $I = 0.034$	pH = 10.34 I = 0.024	pK ₂ (25°)
230	1.676	1.465	1.372	10.67
240	1.374	1.213	1.138	10.63
280	0.342	0.417	0.455	10.56
330	0.737	0.607	0.557	10.77
			me	an = 10.66

The higher value of pK_2 for *cis*-coumarinic acid compared with pK_2 of its *trans*-isomer (9.63) ⁵ is reasonable. In the *cis*-isomer there is a lower mesomeric effect



(shown also by lower ϵ and lower λ_{max} of absorption bands), and a consequent decrease in acid strength of the phenolic —OH (i.e. higher pK₂). At the same time this means



less competitive mesomeric effect in so far as the —OH of the carboxyl group is concerned. Hence an increase of the acid strength of —COOH (lower pK_1) results. The pK_1 of *cis*-coumarinic acid cannot be determined because it lactonizes in acidic pH to coumarin. However, this reasoning is justified by the fact that ΔpK_1 (*trans-cis*) = + 0.56 6

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for unsubstituted cinnamic acid. It is probable that higher ΔpK_2 (*cis-trans* = + 1.034) for coumarinic acid results because of the hydrogen bonding, possible only in the *cis*-isomer, thereby further reducing the acid strength of the phenolic —OH.

The data in fig. 3 show that borate enters into complex formation with the *cis*-coumarinic acid anion (HA⁻) and not with the doubly ionized ion (A²⁻). The complex, formulated as



cannot be formed with the *trans*-isomer of HA⁻. This has been verified experimentally. Patzak and Neugebauer ³ report that *cis*-coumarinic acid on irradiation is transformed into the *trans*-isomer and they claim to have isolated the *trans*-isomer from irradiated solutions. Normally *trans*-compounds isomerize to *cis*- on irradiation because of the extra energy required to overcome the repulsive force of the *ortho*-hydrogens in the *cis*-form.



The parent compound, cinnamic acid, also behaves in the normal manner. The data given in fig. 4 show that in addition to *cis-trans* isomerization, as reported by Patzak, there is photolysis or some other photochemical transformation on irradiation. The *cis*-coumarinic acid anion (A^{2-}) was irradiated by a 125 W Wood's lamp for over an hour. The absorption spectrum of the irradiated solution, however, differs very much from that of the *trans*-isomer (A^{2-}). Had there been incomplete transformation of *cis* to *trans* and no photolysis, the curve 3 (fig. 4) ought to have passed through an isobestic point at 335 m μ where curves 2 and 6 cross. The u.-v. spectrum of the irradiated solution on acidification is also much different from that of the *trans*-isomer molecule (H₂A).

APPENDIX

Considering the equilibria (1), (2) and (5), it can be shown that

$$D_{n} = \frac{[D_{1} + (D_{2}K_{h})/(a_{n}f_{2}) + (D_{3}K_{2}K_{h})/(a_{n}^{2}f_{3}) + (D_{4}K_{d}K_{h}^{2}K_{2}c_{1})/(a_{n}^{2}f_{3})]}{[1 + K_{h}/(a_{n}f_{2}) + (K_{2}K_{h})/(a_{n}^{2}f_{3}) + (K_{d}K_{h}^{2}K_{2}c_{1})/(a_{n}^{2}f_{3})]},$$
(8)

where $K_d = (K'_d/K_w)$, K_w being the ionic product of water. This equation can be solved for 5 unknowns utilizing data for 5 different pHs, provided the free coumarin concentration c_1 is known in each case. It may be worthwhile to attempt to do this by polarography. Otherwise c_1 can be eliminated from eqn. (8) by employing data at two different wavelengths for the same pH. This, however, increases considerably the number of unknowns for which the final sets of equations have to be solved and renders the calculations very laborious.

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¹ Mattoo, Trans. Faraday Soc., 1956, 52, 1184.

² Harle and Lyons, J. Chem. Soc., 1950, 1575.

³ Patzak and Neugebauer, Monatsh., 1951, 82, 662

⁴ Mattoo, Trans. Faraday Soc., 1956, 52, 1462.

⁵ Mattoo, unpublished data.

⁶ Dippy and Page, J. Chem. Soc., 1938, 357.

7 Davies, J. Chem. Soc., 1938, 2093.

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