Modification of Reaction Rates by Complex Formation. V. Alkaline Hydrolysis of Some Xanthine Complexes of Acetoxycinnamic Acids and Related Compounds[1]

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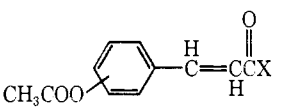
Abstract: The kinetics of alkaline hydrolysis of some ring-substituted acetoxycinnamic acids and related compounds were studied in the presence of theophylline anion and substituted theophyllines. Inhibition of the rate of hydrolysis by these xanthines was attributed to complex formation between the cinnamates and the xanthines. q_{11} values (q_{11} is the fractional decrease in reactivity of the complexed substrate relative to the free substrate) of about 0.4 were found for several systems. These are the first reported q_{11} values substantially smaller than unity for the attack of hydroxide ion on a complexed ester. They are interpreted to mean that the xanthine molecule is not located close enough, in the complex, to the reacting ester group to affect the rate sterically, but exerts only a minor polar effect. 7-(2,3-Dihydroxypropyl)theophylline catalyzes the hydrolysis of acetoxycinnamates; on the basis of structural variations in the xanthine and kinetic study of the catalysis it appears that the catalysis proceeds not *via* a complex but in an intermolecular manner. The region of molecular overlap in cinnamate–xanthine complexes is further defined by these low q_{11} values.

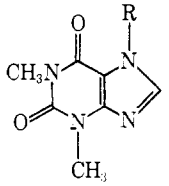
To learn more about the molecular overlap and the transmission of electronic effects within molecular complexes, the kinetic method for studying complexes[2-4] has been applied to some ring-substituted *trans*-cinnamic acid derivatives. Acetoxy groups substituted on the aromatic ring of these substrates (structures **1a**, **b**, **c**) have been subjected to alkaline hydrolysis in the presence of the ligands **2a–e**. The quantities generated by this technique are K_{11}', the apparent 1:1 complex stability constant, and q_{11}, the fractional decrease in reactivity of the substrate in the complex.



1a, X = OH (*o*-, *m*-, *p*-acetoxycinnamic acids)

b, X = OCH_3 (methyl *o*-, *m*-, *p*-acetoxycinnamates)

c, X = CH_3 (*p*-acetoxybenzalacetone)



2a, R = H (theophylline)

b, R = $CH_2CH_2CH_3$ (7-(*n*-propyl) theophylline)

c, R = $CH_2CHOHCH_3$ (7-(2-hydroxypropyl) theophylline)

d, R = $CH_2CH_2CH_2OH$ (7-(3-hydroxypropyl) theophylline)

e, R = $CH_2CHOHCH_2OH$ (7-(2,3-dihydroxypropyl) theophylline)

Experimental Section

Materials. *o*-Acetoxy-*trans*-cinnamic acid was prepared by heating *o*-hydroxycinnamic acid (K & K Laboratories) with sodium acetate and acetic anhydride for 2 hr on a steam bath. The ester was recrystallized from water and from ethanol; mp 145° (lit.[5] mp 146°). *m*-Acetoxy-*trans*-cinnamic acid was prepared by treating *m*-hydroxycinnamic acid (Aldrich Chemical Co.) in 15 *M* sodium hydroxide with acetic anhydride. The ester was recrystallized from ethanol and from water, mp 151–151.5° (lit.[6] mp 151°). *p*-Acetoxy-*trans*-cinnamic acid was similarly prepared from *p*-hydroxycinnamic acid (K & K) and was recrystallized from aqueous ethanol; sublimes 195° (lit.[7] sublimes 196°).

Methyl *p*-acetoxy-*trans*-cinnamate was synthesized in two steps: first methyl *p*-hydroxycinnamate was prepared by refluxing *p*-hydroxycinnamic acid with methanol in the presence of sulfuric acid for 10 hr; this monoester was recrystallized from water, mp 136.5–137° (lit.[8] mp 137°). This compound was treated, in 15 *M* sodium hydroxide, with acetic anhydride. The product, methyl *p*-acetoxycinnamate, was recrystallized from water, mp 82–83°. *Anal.* Calcd for $C_{12}H_{12}O_4$: C, 65.45; H, 5.49. Found: C, 65.51; H, 5.48. The *ortho* and *meta* compounds were similarly prepared. Methyl *m*-acetoxy-*trans*-cinnamate was recrystallized from aqueous ethanol, mp 60–62°. *Anal.* Calcd for $C_{12}H_{12}O_4$: C, 65.45; H, 5.49. Found: C, 65.44; H, 5.49. Methyl *o*-acetoxy-*trans*-cinnamate was recrystallized from aqueous ethanol, mp 42–43.5°. *Anal.* Calcd for $C_{12}H_{12}O_4$: C, 65.45; H, 5.49. Found: C, 65.50; H, 5.50.

p-Hydroxybenzalacetone was synthesized according to the procedure of Buck and Heilbron[9] and was recrystallized from water, mp 102–103° (lit.[10] mp 102–103°). *p*-Acetoxybenzalacetone was obtained by treating *p*-hydroxybenzalacetone in 15 *M* sodium hydroxide with acetic anhydride. The ester was recrystallized from aqueous methanol, mp 67.5–68.5°. *Anal.* Calcd for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92. Found: C, 70.73; H, 5.91.

Phenyl acetate (Aldrich) was distilled; the fraction boiling at 194–197° was used (lit.[11] bp 194°). *p*-Acetylphenyl acetate was prepared according to the procedure of Chattaway[12] (as were the following four substituted phenyl acetates) and was recrystallized from aqueous ethanol, mp 45–46.5° (lit.[13] mp 54°). *p*-Chlorophenyl acetate was purified by distillation, bp 122° (3 mm) (lit.[14] bp 226–228°). *p*-Methylphenyl acetate was distilled; bp 60° (1 mm) (lit.[14] bp 212–213°). *o*-Nitrophenyl acetate was recrystallized from ethanol, mp 38–38.5° (lit.[15] mp 36–38°). *p*-Nitrophenyl

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(6) F. Reiche, *ibid.*, 22, 2356 (1889).
(7) F. Tiemann and H. Herzfeld, *ibid.*, 10, 63 (1877).
(8) T. Zincke and F. Leisse, *Ann.*, 322, 224 (1902).
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(10) T. Zincke and G. Mühlhausen, *Ber.*, **36**, 134 (1903).
(11) J. M. A. Hoeflake, *Rec. Trav. Chim. Pays-Bas*, **36**, 30 (1916).
(12) F. D. Chattaway, *J. Chem. Soc.*, 2495 (1931).
(13) F. M. Irvine and R. Robinson, *ibid.*, 2091 (1927).
(14) F. Fischer and A. Bürgin, *Pharm. Acta Helv.*, 31, 518 (1956).
(15) L. C. Galatis, *J. Amer. Chem. Soc.*, **69**, 2062 (1947).

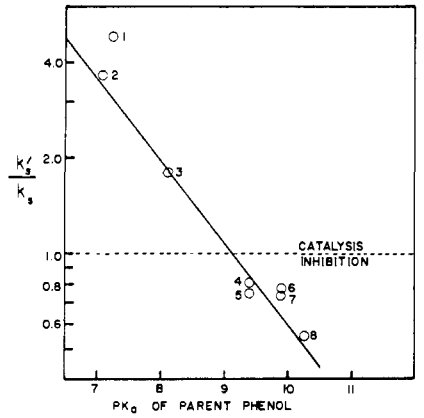

Figure 1. The relative rates of hydrolysis of some substituted phenyl acetates in the absence and presence of 0.1 *M* theophyllinate as a function of the pK_a of the leaving group: pH 10.50; ionic strength 0.3; 1.6% acetonitrile; 25.0°; 1, *p*-nitrophenyl acetate; 2, *o*-nitrophenyl acetate; 3, *p*-acetylphenyl acetate; 4, *p*-acetoxycinnamic acid anion; 5, *p*-chlorophenyl acetate; 6, *m*-acetoxycinnamic acid anion; 7, *o*-acetoxycinnamic acid anion; 8, *p*-methylphenyl acetate.

acetate was recrystallized from Skellysolve B, mp 78° (lit.[15] mp 82°).

Theophylline has been described earlier.[16] 7-(2,3-Dihydroxypropyl)theophylline (Aldrich Chemical Co.) was recrystallized from ethanol, mp 164–165° (lit.[14] mp 155°). This compound displayed no ultraviolet spectral change after 16 hr in pH 10.85 aqueous solution.

7-(*n*-Propyl)theophylline was prepared by a modified Schwabe process:[17] 0.277 mole of theophylline was added to a flask containing 0.277 mole of potassium hydroxide dissolved in 175 ml of ethanol. The flask was fitted with a condenser and heated, with the addition of the minimal amount of water required to effect solution. *n*-Propyl iodide (0.30 mole), which had been decolorized by extraction with an aqueous sodium thiosulfate solution, was added and the mixture was refluxed for 6 hr. The solution was evaporated to dryness on a rotary evaporator and the residue was extracted with hot chloroform. After evaporating the chloroform, the compound was recrystallized from boiling water, mp 99–102° (lit.[17] mp 99–100°).

7-(3-Hydroxytheophylline) was prepared by bringing to boiling a mixture of 0.75 mole of theophylline, 1.125 mole of 3-chloro-1-propanol (Aldrich), and 375 ml of water. The boiling solution was treated with a solution of 1.125 moles of sodium hydroxide in 90 ml of water, added over a 3-hr period. After the addition of sodium hydroxide was complete, refluxing was continued for 1 hr. The mixture was evaporated to dryness and the residue was extracted with 750 ml of boiling ethanol; this solution was filtered. The solid that crystallized out was recrystallized twice from anhydrous ethanol, mp 150–151.5° (lit.[18] mp 149–150°).

7-(2-Hydroxypropyl)theophylline was synthesized according to Zelnick, *et al.*[19] Theophylline (0.3 mole) was dissolved in 175 ml of water containing 0.3 mole of sodium hydroxide. 1-Chloro-2 propanol (23 ml) (Eastman)[20] was added and the mixture was refluxed 7 hr. It was evaporated to dryness and the residue was extracted with hot absolute ethanol. The solid that crystallized out upon standing required at least twelve recrystallizations from ethanol, mp 132.5–133.5° (lit.[19] mp 135–136).

The purity of the 7-substituted theophyllines was checked by thin layer chromatography on silica gel with methanol–chloroform (10:90) solvent. R_f values compared favorably with literature values.[22] Nuclear magnetic resonance spectra were in accord with the structures expected.

Second-order rate constants for the alkaline hydrolysis of the acetoxy group (under the conditions described in the following section) are, in M^{-1} sec^{-1}: *o*-acetoxycinnamic acid, 1.64; *m*-acetoxycinnamic acid, 2.33; *p*-acetoxycinnamic acid, 2.60 (these three are actually for the corresponding carboxylate anions); methyl *p*-acetoxycinnamate, 3.90; *p*-acetoxybenzalacetone, 3.71; phenyl acetate, 1.75; *p*-acetylphenyl acetate, 5.46; *p*-chlorophenyl acetate, 2.38; *p*-methylphenyl acetate, 0.90; *o*-nitrophenyl acetate, 9.69; *p*-nitrophenyl acetate, 12.32. Spectrophotometric determination of the dissociation constants (at 25.0°) of the *o*-, *m*-, and *p*-hydroxycinnamic acids gave these values: *o*-hydroxycinnamic acid, $pK_1 = 4.46$, $pK_2 = 9.89$; *m*-hydroxycinnamic acid, $pK_1 = 4.34$, $pK_2 = 9.96$; *p*-hydroxycinnamic acid, $pK_1 = 4.35$, $pK_2 = 9.41$. Numerous ultraviolet absorption spectral data on these substrates and ligands are recorded elsewhere.[23]

Apparatus and Procedures. Temperature control, pH measurements, and spectrophotometric analyses were made with equipment and techniques described earlier.[1,2,16] Stability constants were measured by the kinetic method,[1,2,4] with supplementary spectral studies being used when possible. Solubility studies of complex stability could not be carried out because of the relative instability of aromatic acetates. All studies were at 25.0° in aqueous solution containing 1.6% acetonitrile, at 0.30 *M* ionic strength, with pH maintained constant by carbonate or hydroxide buffers. Weighted least-squares analyses[2] of kinetic data to yield K_{11} and q_{11} values were performed on a Control Data Corporation 1604 digital computer.

Results

Complex Reactivity and Substrate Structure. The alkaline hydrolysis of the acetoxycinnamic acid anions in the presence of theophyllinate was monitored directly in the spectrophotometer cell at pH 11.8–12.0.[24] These quantities were evaluated: for the *o*-acetoxycinnamic acid anion–theophyllinate complex, $K_{11}' = 14\ M^{-1}$; *m*-acetoxycinnamic acid anion–theophyllinate, $K_{11}' = 11\ M^{-1}$; *p*-acetoxycinnamic acid anion–theophyllinate, $K_{11}' = 7.5\ M^{-1}$; the q_{11} values for all three systems were 0.4 ± 0.1. Because of the small stability constants and low q_{11} values, the observed rate inhibitions were small. The reproducibility of stability constants is 10–15% for the *ortho* and *para* compounds, and 25–30% for the *meta* compound. The low q_{11} value, 0.4, could conceivably have been a spurious consequence of a higher q_{11} value superimposed on a concurrent catalysis (by the nucleophile theophyllinate). The nucleophilic reactivity of theophyllinate toward some monosubstituted phenyl acetates was therefore studied. Figure 1 shows the relative rates of hydrolysis of these esters in the absence (k_s) and the presence (k_s') of 0.1 *M* theophyllinate. Evidently appreciable catalysis occurs for esters with good leaving groups. When the pK_a of the parent phenol is greater than 9, apparently little catalysis occurs, and in fact the inhibitions observed for *p*-chlorophenyl acetate were unexpected. A kinetic complexing study was made of the *p*-chlorophenyl acetate–theophyllinate system; this yielded $K_{11}' = 4.7\ M^{-1}$ and $q_{11} = 1$. Thus these simple phenyl acetates appear to form weak complexes, and attack

(16) J. A. Mollica, Jr., and K. A. Connors, *J. Amer. Chem. Soc.*, **89**, 308 (1967).

(17) W. Schwabe, *Archiv Pharm.*, **245**, 323 (1906).

(18) Gane's Chemical Works, Inc., British Patent 756,594 (1956).

(19) R. Zelnick, M. Pesson, and M. Polonovski, *Bull. Soc. Chim. Fr.*, 1773 (1956).

(20) This material is contaminated with 25% 2-chloro-1-propanol. Rather than subject the alcohol to repeated fractional distillations,[21] the solid product was purified.

(21) A. Dewael, *Bull. Soc. Chim. Belg.*, **39**, 395 (1930); W. Fickett, H. K. Garner, and H. J. Lucas, *J. Amer. Chem. Soc.*, **73**, 5063 (1951).

(22) G. L. Szendy, *Arch. Pharm.*, **299**, 527 (1966); W. Schunack, E. Mutschlen, and H. Rochelmeyer, *Deut. Apotheker-Ztg.*, 105, 1551 (1965).

(23) H. Stelmach, Ph.D. Dissertation, University of Wisconsin, Madison, Wis., 1969.

(24) Some substituted cinnamic acids have been shown to undergo pH-dependent decarboxylation. The half-life of *p*-hydroxycinnamic acid at 100° and pH 11.8 is about 173 hr,[25] hence decarboxylation of the product does not occur to a significant extent under our conditions.

(25) L. A. Cohen and W. M. Jones, *J. Amer. Chem. Soc.*, **82**, 1907 (1960).

Table I. Per Cent Change in Rate of Hydrolysis of Methyl Acetoxycinnamates in Presence of Some Xanthines[a]

Ligand	Methyl acetoxycinnamate *ortho*	*meta*	*para*
7-(*n*-Propyl)theophylline	−37	−47	−40
7-(2-Hydroxypropyl)theophylline	+6	−10	−22
7-(3-Hydroxypropyl)theophylline	−12	−25	−25
7-(2,3-Dihydroxypropyl)theophylline	+46	+37	+40
Propylene glycol	+10	+10	+8

[a] pH 10.8 carbonate buffer; ionic strength 0.3; 1.6% acetonitrile; 25.0°. All ligand concentrations 0.2 *M*.

tion in an "intra-complex" catalysis, this could fix the mutual orientation of substrate and ligand in the complex structure. The most obvious alternative catalytic route is a simple intermolecular process, such as nucleophilic attack by a hydroxy group.

Table I gives the results of "one-point" rate studies with obvious structural analogs of 7-(2,3-dihydroxypropyl)theophylline. The significant results from Table I are (1) 7-(2,3-dihydroxypropyl)theophylline has about the same catalytic effect on the *ortho*-, *meta*-, and *para*-substituted substrates; (2) the catalysis is

Table II. Summary of Complexing Data

Substrate	Ligand	Spectral data K_{11}', M^{-1}	Kinetic data K_{11}', M^{-1}	Kinetic data q_{11}
o-Acetoxycinnamic acid anion	Theophyllinate		14	0.4
m-Acetoxycinnamic acid anion	Theophyllinate		11	0.4
p-Acetoxycinnamic acid anion	Theophyllinate		7.5	0.4
p-Acetoxybenzalacetone	Theophyllinate		7	0.4
Methyl *p*-acetoxycinnamate	Theophyllinate		8	0.5
Methyl *p*-acetoxycinnamate	Theophylline	14		
Methyl *p*-acetoxycinnamate	7-(*n*-Propyl)theophylline	20	10	0.7
Methyl *p*-acetoxycinnamate	7-(2-Hydroxypropyl)theophylline		5	0.6
Methyl *p*-acetoxycinnamate	7-(3-Hydroxypropyl)theophylline		5	0.4
Methyl *p*-acetoxycinnamate	7-(2,3-Dihydroxypropyl)-theophylline	11	2	
p-Acetoxybenzalacetone	7-(2,3-Dihydroxypropyl)-theophylline	6	2	
p-Acetoxycinnamic acid anion	7-(2,3-Dihydroxypropyl)-theophylline	4		

by hydroxide on the complex is essentially completely blocked (this phenomenon may also occur with the phenyl acetates possessing good leaving groups, but it is obscured by the catalysis); the acetoxycinnamates, however, possess a side-chain site for complexing, giving significantly smaller q_{11} values.

The stability constants observed for complexation between the acetoxycinnamic acid anions and theophyllinate may be compared with that for *trans*-cinnamic acid anion with theophyllinate,[1] 7 M^{-1}. Taking into account the experimental uncertainties, it appears that the *m*- and *p*-acetoxycinnamic acid anions form theophyllinate complexes of about the same stability as the unsubstituted cinnamate; the *ortho* compound seems to give a significantly larger constant.

Similar kinetic studies of the effect of theophyllinate gave $K_{11}' = 7\ M^{-1}$ and $q_{11} = 0.4$ for the substrate *p*-acetoxybenzalacetone and $K_{11}' = 8\ M^{-1}$, $q_{11} = 0.5$ for methyl *p*-acetoxycinnamate. These neutral substrates behave about the same as the anions.[26]

Complex Reactivity and Ligand Structure. An attempt was made to decrease the experimental uncertainties by choosing a system with a larger stability constant. Numerous xanthines were screened with the preceding substrates. This screening process did not yield any better ligands for this purpose, but it resulted in one unexpected finding, namely, a significant level of catalysis, rather than the expected inhibition, by 7-(2,3-dihydroxypropyl)theophylline, **2e**. This substance catalyzed the hydrolysis of all of the acetoxycinnamate substrates. The mode of catalysis was of interest because, if it occurs with preliminary complex formation negligible or absent in the structural analogs of the dihydroxy compound; (3) the presence of the glycol function itself cannot account for the full level of catalysis.

Table II lists all K_{11}' and q_{11} values obtained in this work. These points summarize the experimental findings bearing on the catalytic phenomenon: (1) 7-(2,3-dihydroxypropyl)theophylline catalyzes the hydrolysis of the acetoxy substrates at high pH; (2) at pH 8.5, 7-(2,3-dihydroxypropyl)theophylline inhibits the hydrolysis; (3) 7-(*n*-propyl)-, 7-(2-hydroxypropyl)-, and 7-(3-hydroxypropyl)theophyllines all inhibit the hydrolysis under the same conditions at which the 2,3-dihydroxy ligand causes catalysis; (4) K_{11}' values for a substrate with 7-(2,3-dihydroxypropyl)-theophylline differ markedly when determined by the spectral and kinetic methods.

A kinetic analysis of the alternate reaction schemes (the intracomplex and the intermolecular catalysis),[23] combined with the nonspecificity with respect to ring position of the acetoxy group (Table I), inability of the monohydroxy theophyllines to effect catalysis, and the pH effect on the catalysis, indicates that the catalysis does not proceed *via* a complex. It may involve a nucleophilic attack by an anion of the 7-(2,3-dihydroxypropyl)theophylline (or the kinetic equivalent). pK_a values for some model alcohols[27] are consistent with this suggestion: 1-propanol, 16.3; 2-propanol, 16.8; propylene glycol, 14.7.

Discussion

The q_{11} values of 0.4 for complexes of theophylline anion with several acetoxycinnamic acid derivatives

(26) The stability constant for the complex of methyl *p*-acetoxycinnamate with neutral theophylline, determined spectrally, was 14 M^{-1}.

(27) J. Hine and M. Hine, *J. Amer. Chem. Soc.*, 74, 5266 (1952); P. Ballinger and F. A. Long, *ibid.*, 82, 795 (1960).

are the first reported that are substantially smaller than unity for attack by hydroxide ion on an ester. A q_{11} of 0.4 represents, at 25°, an increase in free energy of activation of only 0.3 kcal/mol. For the alkaline hydrolysis of esters (such as methyl *trans*-cinnamate) complexed with xanthines, q_{11} values approaching unity have been consistently observed.[2,3] These have been interpreted in terms of ground-state stabilization (blockage of one side of the ester from attack by the nucleophile) and increase in the transition state energy (by restricting the transformation of the planar ester group to the nonplanar tetrahedral intermediate).[2] Both phenomena are essentially steric effects, though further influence might be exerted by polar effects of the ligand on the substrate. The simplest interpretation of the present q_{11}'s of 0.4 is that, in the complex, the ligand is not situated closely enough to the reacting acetoxy group to cause any steric effects; the inhibition observed is thus the consequence of a small polar effect.

This interpretation is consistent with all related information. Cinnamate–xanthine complexes probably form with the molecular planes parallel.[1] Molecular models and X-ray structural data[28] indicate that an acetoxy group will lie out of the plane of the aromatic ring to which it is bonded. In the plane-to-plane complex the acetoxy group is still free to undergo attack by hydroxide and subsequent transformation to the intermediate and products, with only minor perturbations of an electronic nature. Even the *o*-acetoxy compound seems free of steric blockage by a ligand molecule situated appropriately on the substrate. To be consistent with the high q_{11} values for attack at the methyl ester end of such substrates, the xanthine must be located so that it is very near the carboxyl carbon as well as the phenyl ring.[29] Substantial modifications in the basic cinnamate structure (ionization, esterification, ring substitution) have introduced no inconsistent patterns. Thus this work supports the working assumption that it is reasonable, in a first approximation, to think of *a complex structure* when comparing similar substrates and ligands. The results thus far do not indicate the relative orientation of the "ends" of the cinnamate and xanthine molecules; that is, we cannot yet specify whether the five- or the six-membered ring of the xanthine is lying closer to the phenyl ring of the cinnamate. However, the demonstration of q_{11} values of about 0.4 with these acetoxy substrates further limits the conceivable structures for these complexes.

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(28) A. W. Hanson, *Acta Cryst.*, 18 599 (1965); M. Sax and R. Desiderato, *ibid.*, 23 319 (1967).

(29) We have not ruled out the possibility, however, that the relative orientation of substrate and ligand may change significantly under the influence of the approaching nucleophile.