Studies of reactions within molecular complexes: alkaline hydrolysis of substituted phenyl benzoates in the presence of xanthines

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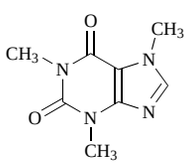
Complexation with caffeine and theophylline-7-acetate depresses the rate of alkaline hydrolysis of substituted phenyl benzoates and is consistent with the formation of molecular complexes with 1 : 1 stoichiometry between the hosts and esters; stacking of the xanthines is excluded as an explanation in the range of concentrations studied. Brønsted-type correlations have been determined for the rate and complexation constants and for the transition-state binding constants. Development of effective charge in the transition state of the reactions in bulk solvent is slightly less than that in the host–ester complex, consistent with a similar electronic environment in both states. The negative Brønsted β values for K_S indicate that the interactions between ester and hosts involve electron donation to the host from the ester. Inhibition of hydrolysis is attributed to repulsion of the hydroxide ion from the host–ester complex by the extra hydrophobicity engendered by the xanthine host, as well as by the weaker binding of the transition state to the host compared with that in the host–ester complex.

Introduction

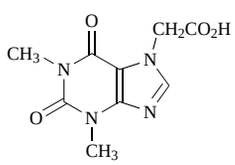
Interactions between the π orbitals of heteroaromatic bases, together with hydrogen bonds, are important forces which stabilise the helical structure of deoxyribonucleic acid (DNA). Since flat aromatic molecules are known to intercalate between the base pairs of DNA by means of π-π interactions, model studies of π-π complexes could have a significant importance in understanding enzymatic reactions and protein binding processes.

Higuchi and Lachman[1] found that caffeine, which possesses a similar molecular structure to those of nucleotide bases, decreases the rate of hydrolysis of benzocaine (ethyl 4-amino-benzoate) due to the formation of a less reactive molecular complex. Menger and Bender[2] observed similar rate retardation in the hydrolysis of *N*-(indole-3-acryloyl)imidazole, *p*-nitrophenyl 3-indolylacetate and *p*-nitrophenyl 3-indolylacrylate in the presence of 3,5-dinitrobenzoate. It was concluded that the decrease in reactivity is due to charge-transfer interaction stabilizing the ground state of the complexed species. The effect of imidazoles, purines and xanthines was also investigated on the hydrolysis of *trans*-methylcinnamate and a broad range of aromatic esters by Connors and co-workers,[3] who found that xanthines form more stable complexes than imidazoles and purines.[3a] In the case of caffeine, complexation appeared to involve more than one caffeine molecule per ester. Interestingly, they found that theophylline anion catalyses the hydrolysis rate of acetates with good leaving groups and inhibits the hydrolysis rate of acetates with poor leaving groups.[3c]

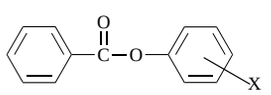
To study the origin of the effect of purine derivatives on the reactivity of aromatic esters using the hydrolysis of benzocaine or other alkyl esters is difficult because of problems of accuracy associated with the analysis of the rate constant. Such relatively imprecise data are not able to distinguish between 1 : 1 and 1 : 2 complexation, as would occur in stacking of the purine bases. We decided to investigate this question using the hydrolysis of phenyl esters because the rate constants of these can be followed very accurately by UV–VIS spectrophotometry. Accurate rate constants make it possible to obtain information on the microscopic medium within the host–ester complex from the effect of polar substituents on reactivity of the complex. We may estimate the relative stability of the ground and transition states of complexes by applying transition state binding theory.[4] Electronic information about the states can also be obtained from Brønsted's β values.[5] The alkaline hydrolysis of a range of substituted phenyl benzoates was studied in the presence and absence of caffeine and theophylline-7-acetic acid (TAA). TAA was chosen because it has a negative charge, preventing self-aggregation at pH values where the reactivity of esters is studied.



Caffeine (**1**)



Theophylline-7-acetic acid (TAA) (**2**)



Substituted phenyl benzoates (**3**)

3a X = 4-NO_2
b X = 3-NO_2
c X = 4-Ac
d X = 3-Cl
e X = 4-Cl
f X = H
g X = 3-Me

Experimental

Materials

The materials used for kinetics were of analytical grade or were purified before use. Water was double-distilled from glass and degassed. Acetonitrile (analytical reagent grade) was purged by passage through a silica gel column. Caffeine was recrystallized from ethanol–water, mp 271–272 °C (lit.,[1] 271–272 °C).

TAA **2** was prepared as follows. Chloroacetic acid (0.01 mol) in water (50 ml) containing $KHCO_3$ (0.01 mol) was added dropwise, with stirring, to a solution of theophylline (0.01 mol) and $KHCO_3$ (0.01 mol) in water (100 ml). The mixture was refluxed for 4 h and then cooled and filtered. The filtrate was acidified with dilute HCl to pH 2 and the precipitate collected by filtration. Recrystallization from ethanol–water gave needles mp 268–270 °C (lit.,[6] mp 269–270 °C) in 85% yield (Calc. for $C_9H_{10}N_4O_4 \cdot H_2O$: C, 45.38; H, 4.23; N, 23.52%. Found: C,

Table 1 Conditions for measuring kinetic parameters for the alkaline hydrolysis of phenyl benzoates in a range of hydroxide concentration in 31% acetonitrile–water at 25 °C

Ester	[**3**]/10^{-4} M[a]	k_{un}/10^{-3} s^{-1}[b]	[OH^-]/10^{-3} M[c]	N[d]	λ/nm[e]	k_{OH}/10^{-3} M^{-1} s^{-1}[f]
3a	0.415	2.87–8.31	3.50–10.0	4	400	820 ± 10
3b	5.00	1.43–4.13	3.50–10.0	4	390	410 ± 5
3c	0.50	1.18–3.30	3.50–10.0	4	325	333 ± 6
3d	5.00	1.50–3.05	10.0–20.0	3	315	150 ± 3
3e	5.00	1.38–2.73	10.0–20.0	3	315	138 ± 2
3f	10.0	2.10–3.15	30.0–50.0	3	310	63.6 ± 1.5
3g	10.0	1.65–2.60	30.0–50.0	3	310	51.5 ± 1.6

[a] Ester concentrations. [b] Range of observed first order rate constants. [c] Range of hydroxide concentrations. [d] Number of data points, not including duplicates. [e] Wavelength employed in the kinetics. [f] Values agree with those determined by Kirsch, Clewell and Simon.[7]

45.16; H, 4.43; N, 23.27%). ^{1}H NMR {270 MHz ([$^2H_6$]DMSO)} δ 8.03 (s, 1H, aromatic imidazolyl ring); 5.07 (s, 2H, CH_2CO_2H); 3.42 and 3.19 (2s, 6H, CH_3N).

The substituted phenyl benzoates were prepared as follows. The substituted phenol (0.01 mol) and benzoyl chloride (0.01 mol) were dissolved in dry pyridine (100 ml), and the mixture was gently warmed for a few minutes. After cooling, the solution was poured into cold water (100 ml). The solid material (in some cases initially an oil) was separated and the product was recrystallized from propan-2-ol. The benzoates were characterized by elemental analysis and by comparison of melting points with literature values.[7]

Methods

Two stock solutions containing the same amount of KOH in 31% acetonitrile–water were prepared; one possessed the xanthine host from which a range of solutions was made up. TAA was neutralized with an equivalent amount of KOH. The pH of the solutions was adjusted to the same value by addition of either dilute KOH or HCl. In order to solubilize the systems at the required host concentrations of up to 0.25 M it was necessary to operate with a solvent with 31% (v/v) acetonitrile–water. The pK_w in 31% acetonitrile–water was determined to be 14.65 by measurement of the pH of solutions of 0.1 M HCl and KOH in 31% acetonitrile–water. Hydroxide ion concentration was obtained from the pH using the equation (pH = log [OH] + 14.65).

Reactions were initiated by the addition of an aliquot (0.01 to 0.05 ml) of a solution of the substrate (acetonitrile) on the tip of a glass rod to 2.5 ml of buffered solution, contained in a 1 cm path length silica cuvette in the thermostatted cell compartment of either a Pye Unicam SP 800 or a Perkin-Elmer Lambda 5 UV/VIS spectrophotometer. A few vertical strokes of the glass rod effected mixing. The reaction was followed by monitoring the change in absorbance with time at the optimal wavelength for analysis of the product. The pH values of the solutions were measured before and after each kinetic run using a Radiometer PHM62 meter with a Russell CMAWL combined electrode, calibrated with BDH buffers. Data for experiments where the pH changed by more than 0.1 units were discarded. The release of 3-Me, phenyl, 4-Cl and 3-Cl phenolate ions were measured at wavelengths above 305 nm since both caffeine and TAA produce UV spectra with maxima between 200–305 nm. Relatively high concentrations of ester were required to obtain good kinetics.

Pseudo-first order rate constants were obtained from linear plots of $\log(A_\infty - A_t)$ *vs.* time where A_t is the absorbance of the solution at the given wavelength at time t. Rate constants were also obtained by fitting the value of A_t (which increases with time in these cases) to the equation $A_t = A_\infty - (A_\infty - A_o) \times \exp(-kt)$.

Data were fitted to theoretical equations by use of programs operating on an Opus IV or V PC or by use of 'grid search' programs written in BASIC and employing a BBC Master 128 computer.

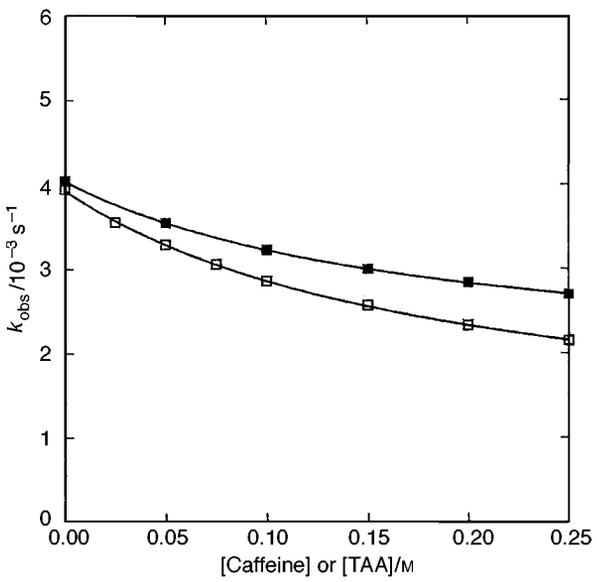
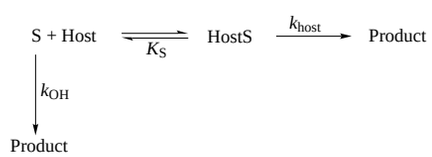


Fig. 1 The dependence of alkaline hydrolysis of *p*-nitrophenyl benzoate on concentrations of caffeine (■) (in 5.0×10^{-3} M KOH) and TAA^- (□) (in 4.75×10^{-3} M KOH) in 31% acetonitrile–water and at 25 °C. Lines are calculated from eqn. (1).

Results

The alkaline hydrolysis of substituted phenyl benzoates obeyed good pseudo-first order kinetic (k_{obs}) up to 90% completion of the reaction. The second order rate constants for the hydrolysis of phenyl benzoates in the absence of host (k_{OH}) were determined from measurements of k_{obs} over a range of hydroxide concentrations (Table 1) by division by hydroxide ion concentration. Pseudo-first order rate constants (k_{obs}) for the hydrolysis of substituted phenyl benzoates in the presence of a series of varying concentrations of caffeine and TAA obey eqn. (1). The complexation between the hosts and the ester is assumed to possess 1 : 1 stoichiometry, as illustrated in Scheme 1.



Scheme 1

The observed pseudo-first order rate constants for the alkaline hydrolysis of **3a** *vs.* concentration of caffeine or TAA are shown in Fig. 1.

The derived parameters (k_{OH}, k_{host} and K_S) from the fit of k_{obs} *vs.* concentration in eqn. (1) for the alkaline cleavage of substituted phenyl benzoates are displayed in Tables 2 and 3. The kinetic parameters k_{OH} and k_{host} (for both caffeine and TAA)

Table 2 Rate and complexation parameters for the alkaline hydrolysis of phenyl benzoates in the presence of caffeine in 31% acetonitrile–water at 25 °C, ionic strength at 0.3 M

Ester[a]	$k_{host}/10^{-3}$ M^{-1} s^{-1}[b]	$K_S/10^{-3}$ M[c]	$\Delta k_{obs}/10^{-3}$ s^{-1}[d]	Δ[**1**]/M[e]	pK_a[f]
3a	340 ± 60	186 ± 5	2.7–4.05	0–0.25	7.14
3b	247 ± 60	136 ± 8	2.1–2.53	0–0.1	8.35
3c	170 ± 90	93 ± 9	1.12–1.51	0–0.1	8.05
3d	64.2 ± 8.0	73 ± 2	1.31–1.96	0–0.1	9.02
3e	104 ± 13	70 ± 6	1.52–1.78	0–0.1	9.39
3f	38.8 ± 6.0	50 ± 3	1.55–2.10	0–0.1	9.95
3g	29.1 ± 7.0	42 ± 4	1.15–1.65	0–0.1	10.09

[a] Ester concentrations as given in Table 1. [b] Second order rate constants for the hydrolysis of benzoate esters inhibited by caffeine. [c] Dissociation constants of caffeine–ester complexes. [d] Range of rate constants; six data points not including duplicate runs. [e] Range of concentrations of caffeine. [f] pK_a of the leaving phenol group.[9]

Table 3 Rate and complexation parameters for the alkaline hydrolysis of phenyl benzoates in the presence of TAA^- in 31% acetonitrile–water at 25 °C, ionic strength 0.3 M

Ester[a]	$k_{host}/10^{-3}$ M^{-1} s^{-1}[b]	$K_S/10^{-3}$ M[c]	$\Delta k_{obs}/10^{-3}$ s^{-1}[d]	Δ[**1**]/M[e]	N[f]
3a	156 ± 13	193 ± 8	2.15–3.95	0–0.25	8
3b	57.9 ± 17	140 ± 14	1.09–2.21	0–0.2	6
3c	51.0 ± 7.0	93 ± 4	1.86–3.33	0–0.1	6
3d	23.0 ± 1.5	91 ± 2	0.834–1.5	0–0.1	6
3e	38.5 ± 5.6	65 ± 8	0.738–1.38	0–0.1	6
3f	9.54 ± 0.22	53 ± 1	0.94–2.11	0–0.1	6
3g	8.40 ± 0.20	43 ± 1	0.64–1.54	0–0.1	6

[a] Ester concentrations as given in Table 1. [b] Second order rate constants for the hydrolysis of benzoate esters inhibited by TAA^-. [c] Dissociation constants of TAA^-–ester complexes. [d] Range of rate constants. [e] Range of concentrations of TAA^-. [f] Number of data points not including duplicates.

Table 4 Dissociation constants for complexes between caffeine and TAA^- and substituted phenyl benzoates in the ground and transition state.[a]

	Caffeine		TAA^-	
Ester	$K_S/10^{-3}$ M	$K_{TS}/10^{-3}$ M	$K_S/10^{-3}$ M	$K_{TS}/10^{-3}$ M
3a	186	466	193	1010
3b	136	206	140	997
3c	93.0	182	93.0	607
3d	73.0	171	91.0	593
3e	70.0	96.6	65.0	233
3f	50.0	82.0	53.0	353
3g	42.0	74.3	43.0	263

[a] K_S values are taken from Tables 2 and 3 and K_{TS} values are calculated as shown in the results section.

and the dissociation constants of the host–ester complexes (K_S) follow Brønsted type eqns. (2)–(6). Dissociation constants, K_{TS},

$$k_{obs}(\text{rate}) = (k_{OH}[K_S] + k_{host}[\text{Host}]_o)[OH^-]/(K_S + [\text{Host}]_o) \quad (1)$$

Alkaline hydrolysis in the absence of **1** *and* **2**:

$$\log k_{OH} = -0.40\ (\pm 0.03)\ pK_a + 2.85\ (\pm 0.30) \quad (r = 0.9829;\ n = 7) \quad (2)$$

with caffeine:

$$\log k_{host} = -0.36\ (\pm 0.02)\ pK_a + 2.07\ (\pm 0.17) \quad (r = 0.9957;\ n = 7) \quad (3)$$

$$\log K_S = -0.20\ (\pm 0.03)\ pK_a + 0.723\ (\pm 0.26) \quad (r = 0.9524;\ n = 7) \quad (4)$$

with TAA:

$$\log k_{host} = -0.43\ (\pm 0.03)\ pK_a + 2.25\ (\pm 0.23) \quad (r = 0.9920;\ n = 7) \quad (5)$$

$$\log K_S = -0.20\ (\pm 0.03)\ pK_a + 0.74\ (\pm 0.28) \quad (r = 0.9438;\ n = 7) \quad (6)$$

of host–transition state complexes were calculated according to the equation ($K_{TS} = k_{OH}K_S/k_{host}$) and are recorded in Table 4; they obey Brønsted-type equations [eqns. (7) and (8)].

Transition state equilibrium constants:
for caffeine:

$$\log K_{TS} = -0.25\ (\pm 0.03)\ pK_a + 1.41\ (\pm 0.27) \quad (r = 0.9665;\ n = 7) \quad (7)$$

for TAA:

$$\log K_{TS} = -0.22\ (\pm 0.06)\ pK_a + 1.62\ (\pm 0.51) \quad (r = 0.8614;\ n = 7) \quad (8)$$

Discussion

Stacking model

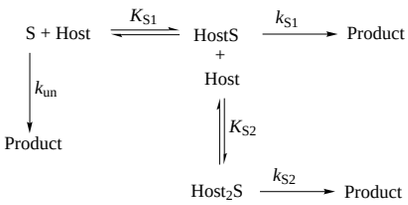
If caffeine forms stacking complexes with multiple stoichiometry,[3] *e.g.* in a 1:2 molar ester to caffeine ratio, then two sides of the ester could be blocked by caffeine in multiple stacking and the ester would be 'sandwiched' and protected against attack by hydroxide ions. In the case of 1:1 complexes caffeine would be expected to block only one side of the ester from nucleophilic attack and thus reduce the rate constant nominally to half. The observed second order rate constants for the hydrolysis of phenyl benzoates in their complexes with caffeine or TAA^- anion indicate that complete inhibition does not occur and the observed pseudo-first order rate constants fit eqn. (1), which is derived from the assumption of 1:1 complex formation. These data are accurate enough to exclude 1:2 and 1:3 complexes. If the complexation has 1:1 and 1:2 stoichiometry then rate constants would follow eqn. (9) derived from Scheme 2.

$$k_{obs} = (k_{OH}K_{S1}K_{S2} + k_{S1}K_{S2}[\text{Host}] + k_{S2}[\text{Host}]^2)[OH^-]/(K_{S1}K_{S2} + K_{S2}[\text{Host}] + [\text{Host}]^2) \quad (9)$$

Data obtained for the hydrolysis of ester in the presence of xanthines do not give significant values of K_{S2} and k_{S2}. For example, parameters obtained from the application of eqn. (9) to the hydrolysis of esters in the presence of caffeine and TAA are given in Table 5 and have substantial error components in the terms corresponding to the second complexation. The value of K_{S1} corresponds to K_S in eqn. (1) and has reasonable error limits. Eqn. (9) would, of course, provide an overall better fit to

Table 5 Parameters obtained from the hydrolysis of ester **3a** in the presence and absence of caffeine (**1**) and TAA (**2**) for 1 : 2 complexation by fitting eqn. (9)

Purine	$K_{OH}/10^{-3}$ M^{-1} s^{-1}	$k_{S1}/10^{-3}$ s^{-1}	$k_{S2}/10^{-7}$ s^{-1}	K_{S1}/M	$K_{S2}/10^{-7}$ M
1	4.05 ± 0.01	1.90 ± 0.30	−1.90 ± 122	0.166 ± 0.03	4.82 ± 2.38
2	3.95 ± 0.01	1.46 ± 0.50	−4.00 ± 0.120	0.143 ± 0.03	2.95 ± 9.08



Scheme 2

the data because there are more disposable parameters than in eqn. (1).

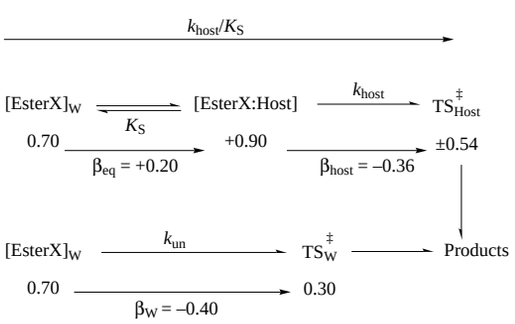
Theophylline-7-acetate anion (TAA^-), which should not aggregate due to electrostatic repulsion, also forms complexes with 1 : 1 stoichiometry, eqn. (1). The reactivity difference observed in the alkaline hydrolysis of benzoates complexed by caffeine and TAA^- may by ascribed to the negative charge of the latter, which electrostatically repels hydroxide ion attack.

We conclude that stacking does not account for the inhibition of ester hydrolysis by xanthine derivatives. Moreover, the heterocyclic host molecules cannot form a cavity, into which a substrate may penetrate and from which hydroxide ion may be excluded.

Charge development

The negative Brønsted β values for the dissociation of the host–ester complexes (K_S) [eqns. (4) and (6)] indicate an increase in positive effective charge on the leaving ester oxygen in the complex compared with that in free aqueous solution. The introduction of a carboxylate group to the host, as in TAA^-, has no observable effect on the effective charge development in the complex.

Effective charges may be derived for the complexation reactions from β values from Brønsted equations and Scheme 3



Scheme 3 Effective charge map for alkaline hydrolysis of phenyl benzoates in the absence and in the presence of caffeine. The numbers represent effective charges in various states, which are relative to the ionization of phenol in water.[5] The β values are quoted with the convention that they are positive for increase in positive effective charge from left to right.

illustrates the effective charge map for the reaction in the presence and absence of caffeine. The map for the interactions of esters with TAA^- ion has similar values. The change in effective charge from free ester to complexed transition state (−0.16) is less negative than from free ester to transition state in aqueous solution (−0.40). The smaller selectivity for the complexation reaction is not consistent with the reactivity–selectivity hypothesis and this can be explained by the fact that the two rate parameters (k_{OH} and k_{host}/K_S) have different units, so that comparison is not possible. Comparison of k_{host} and k_{OH}, the rate constants for attack of hydroxide ion on complexed and free ester, indicates that the selectivities are approximately equal, consistent with relatively similar rate constants (**3a** has k_{OH} = 820, $k_{caffeine}$ = 340 and k_{TAA} = 156 M^{-1} s^{-1}).

The system may be compared with that of the cetyltrimethylammonium bromide catalysed alkaline hydrolysis of esters; there is no substituent effect for the complexation of substituted phenyl laurates with the micelle.[8] In that case it is suggested that the ester function resides in a microenvironment similar to that of the bulk solvent which is not substantially involved in the binding process. Moreover the ester group in that complex is in the Stern layer, to which hydroxide ion is attracted. In the xanthine complexation the host provides a microenvironment for the ester group, which is more electron attracting than that of the bulk solvent.

It is paradoxical that a host which binds the ester by attraction of electrons reduces the reactivity of the ester in the complex towards attack by hydroxide ion. The complexation almost certainly involves a parallel planar arrangement of the two flat molecules and charge polarization towards the host. It may be that the π-electron cloud of the ester is attracted to the xanthine acceptor host; little else could explain the relatively large increase (+0.2) in effective charge on the ether oxygen when the ester is complexed. The carbonyl functions in the xanthine hosts would effectively act to reduce electron density on the aromatic ring, consistent with its acting as an electron acceptor in molecular complexes with esters. The planar arrangement would be disrupted in the transition state by the formation of a tetrahedral structure and this fits the observation of a more weakly bound transition state–host complex (Table 4). We suggest that the reduced reactivity of the host–guest complex to hydroxide ion attack is, in addition, due to repulsion of the hydroxide ion from the relatively hydrophobic complex. The electron attracting mechanism in the xanthine–ester interaction is unlikely to complex the hydroxide ion; no such complexation has been observed and if it were, it would compete with ester binding.

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