

Kinetic and Spectroscopic Characterization of Ternary Complexes by Numerical Fitting Methods. Catalysis of Acyl-Transfer Reactions by a Macrocyclic Azoniacyclophane[†]

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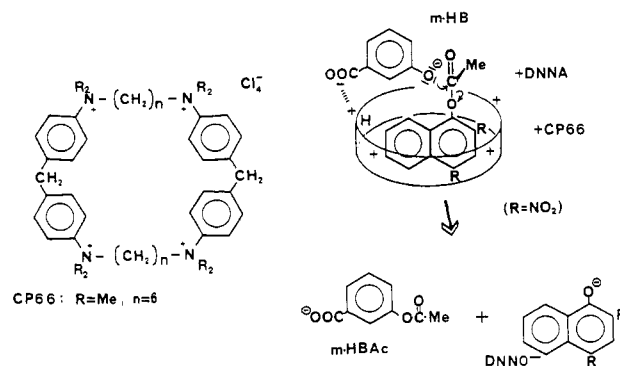
Abstract: Spectroscopic and computational procedures are presented for the analysis of highly complex kinetic schemes occurring in acyl-transfer reactions which are catalyzed by an azoniacyclophane (CP66). The results, supported by product identification and inhibition experiments, indicate an acyl transfer from intracavity-bound substrates such as 2,4-dinitronaphthyl acetate (DNNA) to hydroxybenzoic acids which are bound as cosubstrates to the positively charged CP66 by electrostatic association. The kinetic system is represented by 11 single steps, including the hydrolysis via a binary complex with CP66 and a side reaction to Meisenheimer products. Seven constants are determined by separate measurements with different techniques; three parameters for the ternary complex are then identified by curve fitting to the observed saturation kinetics. A newly developed numerical integration and optimization program allows the description of all parallel reaction parameters including fast equilibria.

Introduction

The formal analysis of artificial catalytic host–guest systems is usually much more complicated than that of biological systems. In the latter, nature has optimized efficiency to a degree that (i) noncatalyzed and other side reactions can usually be neglected, (ii) one can work, e.g., under catalyst (enzyme) saturation, and (iii) well-defined binary and ternary complexes can be assumed. Synthetic biomimetic systems usually do not allow such simplifications which might be the reason why their explicit analysis is until now largely missing.

We want to demonstrate how a combination of different measuring techniques and computer-fitting methods of time–concentration curves by numerical integration can help to identify and to evaluate the many parameters involved in a biomimetic catalytic system which definitely lacks the effectivity but *not* the complexity of an enzymatic process. The investigated reaction of aryl acetates (DNNA and PNPA) with nucleophilic cosubstrates in the presence of the azoniacyclophane CP66 (Scheme I and Chart I) implies the formation of ternary complexes which, besides involving interesting synthetic aspects, leads to further complications in terms of the additional parameters involved. The formation of *ternary complexes* by binding two substrates in a molecular cavity and the thereby attainable increase in reaction rates and selectivity belong to those achievements of nature, for which there are, so far, only few synthetic counterparts. Several known examples of termolecular catalysis¹ require the presence of both substrate and cosubstrate in the cavity, but to the best of our knowledge, no attempts have been reported to analyze in detail the effectivity and the parameters of such synthetic ternary complexes.

Scheme I



Experimental Section

The host compound CP66² and the substrate DNNA^{3a,b} were prepared as described in the literature cited; they and the other commercially available compounds were found by ¹H NMR to be >97% pure.

Kinetic measurements by UV/vis spectroscopy were carried out in thermostatted cuvettes in a Kontron 860 Uvikon instrument with data collection and processing by microcomputers. Absorbances were followed at 438 nm for DNNO[−], at 400 nm for PNPO[−], and at 328 nm for the β-naphthyl acetate reaction. Usually the reactions were followed over 5 half-lives and evaluated by nonlinear least-squares fitting either to the integrated pseudo-first-order equation in the case of noncatalyzed reactions or by CHEMSIM (see below) for saturation kinetics in the presence of CP66 and cosubstrates. The obtained fit with nonlinear correlation coefficients of usually >0.999 indicated statistical errors in rate constants below 0.2% on the average. Almost all rates were run at least in duplicate; the errors reported in Tables I–III (usually <0.5%) are taken from the observed deviations.

Acetonitrile was used for stock solutions and brought to 1.2% (v/v) in the final solutions, showing within ±2% the same rate constants with 4.8% MeCN. The use of dioxane as mediator was abandoned after finding, occasionally, irreproducible results, presumably due to traces of the extreme nucleophile O₂H[−]. All solutions were brought to the desired pH by using the same buffer solutions. The pH was also checked after rate measurements and found to remain constant within ±0.1 unit.

Product Identification. Ester Formation. A 0.05 M borate buffer solution (250 mL, pH = 9.23) with a concentration of β-naphthol of 4.88 × 10^{−4} M was treated at 25 °C with 12.5 mL of the ester solution (4.8% (v/v) MeCN) to give a final concentration of 9.11 × 10^{−5} M DNNA for ~2 half-lives (in the case without CP66, 10.7 min; with CP66

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(1) See, e.g.: (a) Breslow, R.; Overman, L. W. *J. Am. Chem. Soc.* **1970**, *92*, 1075. (b) Murakami, Y.; Ayoma, Y.; Dobashi, K.; Kida, M. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 3633. (c) Sunamoto, J.; Kondo, H.; Okamoto, H.; Terada, O. *Kenkyu Hokoku Nagasaki Daigaku Kogakuba* **1980**, *14*, 103. Sangwan, N. K.; Schneider, H.-J. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1223 and references cited therein.

(2) Schneider, H.-J.; Philippi, K. *Chem. Ber.* **1984**, *117*, 3056. See also: Schneider, H.-J.; Busch, R. *Ibid.* **1986**, *119*, 747.

(3) (a) Schneider, H.-J.; Junker, A. *Chem. Ber.* **1986**, *119*, 2815. (b) Zincke, T.; Krollpfeifer, F. *Lieb. Ann. Chem.* **408**, 311, 1915.

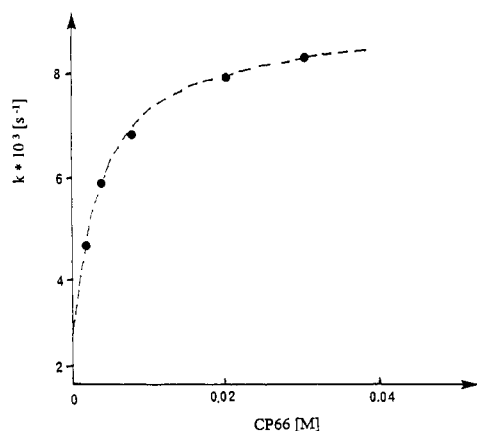


Figure 1. Hydrolysis of DNNA via the binary complex with CP66. Rate constants (s^{-1} , pseudo first order) vs [CP66] with [DNNA] = 2.5×10^{-5} M; measuring conditions and constants from the curve fitting, see Scheme II and text.

(concentration of CP66, 4.4×10^{-3} M), 2.08 min). The mixture was brought to pH 7 and extracted with six 40-mL portions of chloroform, leaving CP66 in the aqueous phase. After drying over $MgSO_4$ and removal of the solvent, the residue was quantitatively analyzed by 1H -NMR integration using dioxane as internal reference; the concentrations relative to the same standard were 6.32×10^{-6} M with and 2.92×10^{-6} M without added CP66. (Due to complexation of DNNA and $DNNO^-$ by excess CP66, absolute yields could not be determined.) The identification of the products was established by the NMR procedure described above and, in addition, following column chromatography over silica gel (with MN silica gel 60, 70–230 mesh ASTM, and chloroform as solvent). The fractions were again analyzed by 1H -NMR comparison; the product assignment was also in accord with thin layer chromatography comparison to authentic samples (Macherey-Nagel Polygram SIL G/UV₂₅₄ and chloroform as solvent).

Meisenheimer Products X1 and X2. The yellow fraction obtained from chromatography as described above showed 1H - and ^{13}C -NMR spectra which agree with either the *ortho* or the *meta* substitution product X₁ or X₂. 1H NMR ($CDCl_3$) (assignments secured by a COSY-45 homoshift-correlated spectrum): δ 8.94 (s, H-1), 8.76 (d, H-2), 8.40 (d, H-5), 7.96 (m, H-3), 7.89 (d, H-9), 7.83 (d, H-13), 7.73 (m, H-4), 7.57 (d, H-10), 7.42 (m, H-11, H-12), 7.31 (d, H-8), 6.91 (s, H-6). ^{13}C NMR ($CDCl_3$): δ 156.1 (C-1), 149.8 (C-12), 143.2 (C-4), 133.9 (C-2), 133.0 (C-7), 130.6 (C-14), 130.2 (C-19), 129.7 (C-20), 129.5 (C-6), 128.4 (C-9), 128.3 (C-10), 127.9 (C-17), 127.1 (C-18, C-15), 125.4, 125.2 (C-8, C-5), 124.1 (C-16), 120.3 (C-3), 117.1 (C-13), 110.2 (C-11).

Equilibrium determinations were carried out as described previously,⁴ usually between 20 and 80% complexation with 7–10 NMR or UV measurements at concentrations suitable for usually 20–80% complexation. The average error in K was $\pm 10\%$ as estimated earlier⁴ for similar titrations.

Numerical simulations were performed with the program CHEMSIM written in PASCAL for the Atari microcomputer, based on stepwise integration of differential equations for k and K parameters which were varied by SIMPLEX routines. The program at the present time allows for the fit of single concentration–time curves separately. Convergence at global minima of square-error sums was secured by choosing several starting values for the optimization, which led to the same, although quite broadly distributed, set of unknown parameters K_6 , k_9 , and k_{10} (Scheme II).

Results and Discussion

We used the easily accessible² tetraazacyclophane derivative⁵ CP66 (Scheme I) as a particularly efficient complexer of naphthalene compounds in aqueous solutions;^{5,6} the presence of four positive charges can lead to a stabilization of negatively charged transition states as well as to a local concentration of hydroxy anions and subsequently to an acceleration of the alkaline

naphthyl acetate hydrolysis.⁷ With the *binary complex* from naphthyl acetate DNNA as substrate and CP66, we observed a maximum rate constant increase of 3.6 (for conditions, see Table I); numerical least-squares curve fitting (Figure 1) furnished the unknown rate constant $k_{cat} = 8.8 \times 10^{-3} s^{-1}$ ($k_{uncat} = 2.32 \times 10^{-3} s^{-1}$) and the association constant for the binary complex $K_A = 325 M^{-1}$.⁸

The addition of hydroxy- or aminobenzoic acids to the binary complex can be expected to lead to a catalyzed acyl transfer from the aryl acetate to the nucleophilic center of the cosubstrate, which is bound by electrostatic attraction between the carboxylate and ammonium groups (see Scheme I). Preliminary attempts using *p*-nitrophenyl acetate (PNPA) as substrate (Chart I, Table II) showed significant effects with hydroxybenzoic acids but considerably larger effects with DNNA as substrate (see below). Since under the reaction conditions the hydrolysis of the acetoxybenzoic acids proceeds substantially slower⁹ than their formation, we observed here the *catalysis of a transesterification* and *not* of a simple hydrolysis.

Reactions of the aryl acetates DNNA and PNPA in the presence of a usually 10-fold excess of 10 different cosubstrates (Tables I, II) yielded the pseudo-first-order rate constants k_{co} without contribution from ternary complexes. Although the pK values of the phenolic groups range around 9.5 (Tables I, II), the pH range to be used was restricted to 9–10 in view of the otherwise too fast, uncatalyzed reactions. Control measurements of k_{un} values for the uncatalyzed reaction at four different pH values between 7 and 10 as well as in the presence of various NaCl concentrations with different ionic strengths secured that rate variations due to changes or errors in pH and ionic strength were smaller than the observed catalytic effects.

For the DNNA ternary-complex reaction, the exploration of suitable cosubstrates involving several naphthalene in addition to benzene derivatives then showed 3,5-dihydroxybenzoic acid (DHBS) to be the most efficient one with 2,7-dihydroxynaphthalene as second (Table I). For the scaling factor f for the efficiency of the ternary-complex catalysis, we use

$$f = (k_{ter} - k_{co}) / (k_{co} - k_{un})$$

where k_{ter} refers to the constant via ternary-complex formation, k_{cp} to the rate catalyzed by CP66 alone, k_{co} to the rate with the cosubstrate alone, and k_{un} to the reaction without cosubstrates and/or CP66, always under the conditions given in Table I. The activation parameters (Table III) as determined from van't Hoff plots for the *net* hydrolysis at pH 9.23 showed, with $\Delta H^\ddagger = 18$ kcal/mol, a much higher temperature dependence than all other reactions ($9 < \Delta H^\ddagger < 13$ kcal/mol). This can be partially due to the (unknown) changes of the pK of the cosubstrates and of the association constants K with temperature and leads to a rather high dependence of the efficiency factor f on temperature, varying, e.g., from $f = 62$ at 328 K to $f = 12$ at 273 K for the reaction of DNNA with DHBS. The low rates observed with 2,3-dihydroxynaphthalene is a consequence of this vicinal diol being only present as a monoanion at the pH of 9.2 which could not be chosen higher in view of the then too fast uncatalyzed reaction. The *negative* f factor obtained in this case suggests a partial blocking of the remaining nucleophilic anionic center by the N^+ charge in CP66. At pH 10, the observed f factors generally were distinctly higher than at pH 9.2 (Tables I, II). This is not unexpected in view of the phenolic pK values which lead to only $< 50\%$ ionization at the lower pH and to subsequently lower efficiency of both binding constants and nucleophilicity in the ternary complexes.

That a large part of the catalyzed DNNA reaction occurs via the ternary complex was also secured by a *product analysis*, which

(4) Schneider, H.-J.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442.

(5) For review, see: Odashima, K.; Koga, K. In *Cyclophanes*; Academic Press: New York, 1983; Vol. II, p 629.

(6) Cf.: Schneider, H.-J.; Philippi, K.; Pöhlmann, J. *Angew. Chem.* **1984**, *96*, 907; *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 908.

(7) Cf.: Tabushi, I.; Kimura, Y. K. *J. Am. Chem. Soc.* **1981**, *103*, 6486.

(8) Due to solubility problems, K_A (K_2 in Scheme II) could not be determined by NMR spectroscopy in this case; its value, however, agrees with data for similar compounds.⁴

(9) Mochida, K.; Matsui, Y.; Ota, Y.; Arakawa, K.; Date, Y. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 3119. Matsui, Y.; Okamoto, A. *Ibid.* **1978**, *51*, 3030.

Table I. Reaction Rate Constants^a of DNNA

cosubstrate	pK _A	$k_{\text{co}} \times 10^4$ (s ⁻¹)	$k_{\text{co}}/\text{CRO}^-$ (L/(mol s))	$k_{\text{ter}} \times 10^4$ (s ⁻¹)	$k_{\text{ter}}/k_{\text{co}}$	<i>f</i>
benzoic acid ^b	4.2	20.0		59.0	2.9	0.3
phenols						
(1) X = H	9.89	13.09	14.5	49.0	3.7	2.6
(1a) X = H ^b		52.0	8.7	214.0	4.1	5.3
(2) X = <i>p</i> -Cl	9.42	16.4 ^c	8.3	80.5	4.9	6.4
(3) X = <i>m</i> -OH	9.44	23.6	11.0	99.8	4.2	4.7
	11.32					
(4) X = <i>m</i> -COOH	4.08	14.1	16.1	79.4	5.6	8.9
	9.9					
(4a) X = <i>m</i> -COOH ^b		60.0	9.1	561.0	9.35	13.5
(5) X = <i>m'</i> -COOH, Y = <i>m</i> -OH	3.84	19.4 ^d	5.7	200.4	10.3	15.7
	9.0					
	10.54					
naphthalenes						
(6) α-OH	9.34	18.7	8.6	90.4 ^e	4.8	5.9
(7) β-OH	9.51	22.3	13.1	111.3	5.0	5.9
(8) 2,3-diOH	8.68	8.7 ^f	2.2	28.2	3.2	-3.4
	12.5					
(9) 2,7-diOH		27.3		226.6	8.3	10.5

^a With CP66 and cosubstrates as noted. (pK values from: Aylward, G. H.; Findlay, T. J. F. *Datensammlung Chemie*, taschentext 27; Verlag Chemie GmbH: Weinheim, 1975. Dean, J. A. *Handbook of Organic Chemistry*; McGraw-Hill Book Company: New York, 1987. Pant, A. N.; Soni, R. N.; Gupta, S. L., *Indian J. Chem.* 1971, 9, 270. Ostacoti, G.; Campi, E.; Gennaro, M. C. *Gazz. Chim. Ital.* 1968, 98, 301. Bartusek, M. *Collect. Czech. Chem. Commun.* 1967, 32, 757.) Rates (errors $\pm 0.5\%$ unless noted otherwise) measured at $25 \pm 0.1^\circ\text{C}$ with 0.05 M borate buffer at pH 9.23 (unless noted otherwise in water with 1.2% acetonitrile); uncatalyzed reaction, $k_{\text{un}} = (8.44 \pm 0.14) \times 10^{-4} \text{ s}^{-1}$; by CP66-catalyzed reaction, $k_{\text{CP66}} = (29.26 \pm 0.33) \times 10^{-4} \text{ s}^{-1}$. Concentrations: [DNNA] = $(4.80 \pm 0.02) \times 10^{-5} \text{ M}$; [CP66] = $(4.47 \pm 0.04) \times 10^{-3} \text{ M}$; [cosubstrate] = $5.00 \times 10^{-4} \text{ M}$, except [resorcinol] = $5.6 \times 10^{-4} \text{ M}$; and ionic strength kept constant at $I = 0.045$ by added NaCl. k_{co} : rate in the presence of cosubstrate. k_{ter} : with cosubstrate and CP66. $k_{\text{co}}/\text{CRO}^-$: k_{co} normalized for cosubstrate concentration. ^b pH = 10.0 with 0.05 M borate buffer; $T = 25 \pm 0.1^\circ\text{C}$; [DNNA] = $2.3 \times 10^{-5} \text{ M}$; uncatalyzed reaction, $k_{\text{un}} = 2.3 \times 10^{-3} \text{ s}^{-1}$; by CP66-catalyzed reaction, $k_{\text{CP66}} = 6.0 \times 10^{-3} \text{ s}^{-1}$; and [benzoic acid] = $2.46 \times 10^{-3} \text{ M}$. ^c ± 0.15 , ^d ± 0.1 , ^e ± 1.1 , ^f ± 0.09 .

Table II. Reaction Rate Constants^a of PNPA

cosubstrate	pK _A	$k_{\text{co}} \times 10^4$ (s ⁻¹)	$k_{\text{co}}/\text{CRO}^-$ (L/(mol s))	$k_{\text{ter}} \times 10^4$ (s ⁻¹)	$k_{\text{ter}}/k_{\text{co}}$	<i>f</i>
phenols						
(1) X = H	9.89	5.4	6.0	6.9	1.3	0.7
(1a) X = H ^b		34.4	3.0	41.7	1.2	1.3
(2) X = <i>p</i> -Cl	9.42	5.9	3.0	7.3	1.2	0.7
(3) X = <i>m</i> -OH	9.44	5.7	1.1	7.70	1.3	1.2
	11.32					
(4) X = <i>m</i> -COOH	4.08	5.1 ^c	5.8	8.1	1.6	2.4
	9.9					
(4a) X = <i>m</i> -COOH ^b		25.6	3.9	37.6	1.5	2.5
(5) X = <i>p</i> -COOH ^b	4.58	23.0	1.4	33.5	1.5	3.1
	9.23					
(6) X = <i>m</i> -OH, Y = <i>m'</i> -COOH	3.84	5.68	1.8	9.4	1.7	2.5
	9.0					
	10.54					
benzoic acids						
(7) X = H ^b	4.2	18.8		21.4	1.1	0.0
(8) X = <i>m</i> -NH ₂ ^b	3.07	20.5		22.6	1.1	0.6
	4.79					
(9) X = <i>p</i> -NH ₂ ^b	2.41	20.0		21.6	1.1	0.0
	4.85					

^a See footnote a to Table I. Errors in $k \pm 1\%$, unless noted otherwise. ^b Uncatalyzed reaction $k_{\text{un}} = (4.31 \pm 0.05) \times 10^{-4} \text{ s}^{-1}$; by CP66-catalyzed reaction, $k_{\text{CP66}} = (6.07 \pm 0.64) \times 10^{-4} \text{ s}^{-1}$. Concentrations: [PNPA] = $(5.02 \pm 0.15) \times 10^{-5} \text{ M}$; [cosubstrate] = $(4.49 \pm 0.02) \times 10^{-4} \text{ M}$; and [CP66] = $(4.49 \pm 0.02) \times 10^{-3} \text{ M}$. k_{co} : rate in the presence of cosubstrate. k_{ter} : with cosubstrate and CP66. $k_{\text{co}}/\text{CRO}^-$: k_{co} normalized for cosubstrate concentration. ^c pH = 10.0 with 0.05 M borate buffer; [PNPA] = $5.0 \times 10^{-5} \text{ M}$; uncatalyzed reaction, $k_{\text{un}} = 1.93 \times 10^{-3} \text{ s}^{-1}$; by CP66 catalyzed reaction, $k_{\text{CP66}} = 2.19 \times 10^{-3} \text{ s}^{-1}$; and [cosubstrate] = $(1.08 \pm 0.1) \times 10^{-3} \text{ M}$, except [phenol] = $2.05 \times 10^{-3} \text{ M}$. ^d ± 0.29 .

showed an increase by >100% of β-naphthyl acetate formation from the cosubstrate in comparison to the same reaction without added cyclophane CP66. As cosubstrate for this preparative study, β-naphthol was chosen in view of the better hydrolytic stability of the resulting ester.¹⁰ Although higher concentrations were needed for this product study, a kinetic check still showed an overall rate constant increase by a factor of 5 compared to results in the absence of CP66.

Another indication for presence and structure of the ternary complex is found by the observed inhibition with 2-naphthalenesulfonic acid (NS). Under the conditions shown in Figure 2, $9.2 \times 10^{-4} \text{ M}$ of the strongly binding NS leads to a rate retardation

with $k_{\text{exp}} = 0.103 \text{ s}^{-1}$. If other and weaker complexes are neglected, one can derive the association constant $K = [\text{complex}]/([\text{NS}][\text{CP66}]) = 5.0 \times 10^3 \text{ M}^{-1}$. This value compares favorably with the constant $K = 6.0 \times 10^3 \text{ M}^{-1}$ found by NMR titration under similar conditions. With naphthalene-2,3-diol as inhibitor in a similar experiment, we observed $K = 1800 \text{ M}^{-1}$.

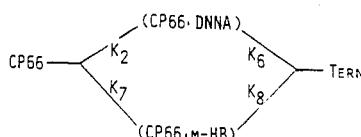
For a full analysis of the ternary-complex reaction with support by all available instrumental techniques, we used the reaction of DNNA in the presence of *m*-hydroxybenzoic acid (*m*-HB) as cosubstrate. 2,4-Dinitronaphthyl acetate (DNNA) was chosen in view of the easy UV detection of the liberated anion (DNNO⁻) but showed a side reaction of up to 30% in the presence of phenolate. Based on similar results with 2,4-dinitronaphthalene and NMR spectra of the products, we assume the formation of

(10) Under the same conditions, we measured for β-naphthyl acetate: $k_{\text{un}} = 7.4 \times 10^{-5} \text{ s}^{-1}$ and $k_{\text{CP66}} = 1.06 \times 10^{-4} \text{ s}^{-1}$.

Scheme II. Participating Reactions with Corresponding Constants^a

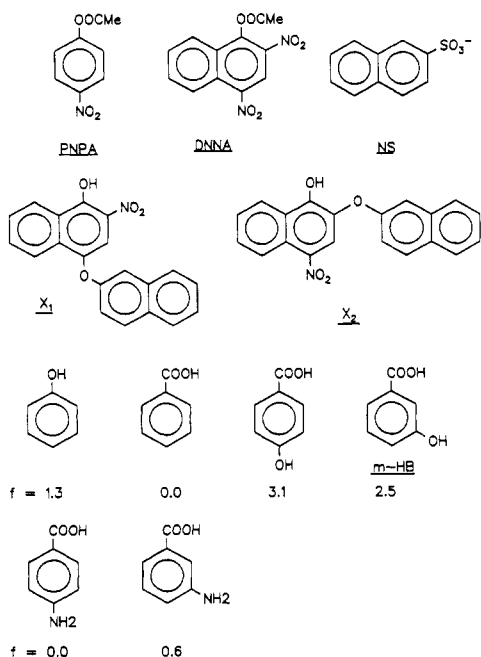
				Method
1	DNNA + OH ⁻	→ DNNO ⁻ + AcOH	$k_1^b = 2.36 \times 10^{-3}$	UV
2	DNNA + CP66	⇌ (CP66*DNNA)	$K_2 = 325$	(From UV-Kin.)
3	(CP66*DNNA) + OH ⁻	→ DNNO ⁻ + AcOH + CP66	$k_3^b = 8.81 \times 10^{-3}$	UV
4	DNNA + m-HB	→ DNNO ⁻ + m-HBAc	$k_4 = 1.4$	UV
5	DNNA + m-HB	→ Meisenheimer (X _{1,2})	$k_5 = 1.7$	UV
6	(CP66*DNNA) + m-HB	⇌ Ter	$K_6 = 10$ (250)	
7	CP66 + m-HB	⇌ (CP66*m-HB)	$K_7 = 280^c$	NMR
8	(CP66*m-HB) + DNNA	⇌ Ter	$K_8 = K_2 * K_6 / K_7$	
9	Ter + OH ⁻	→ DNNO ⁻ + AcOH + CP66	$k_9 = 14.6$ (0.635) ^d	
10	Ter + m-HB	→ X _{1,2} + CP66	$k_{10} = 6.1$ (0.27) ^d	
11	DNNO ⁻ + CP66	⇌ (CP66*DNNO ⁻)	$K_{11} = 2.1 \times 10^{+4}$	UV

Random Mechanism of Ternary Complex Formation



^a Rate constants k in s⁻¹; equilibrium constants K in L/mol; measurements in 5% dioxane at 25 °C; pH = 10 (0.05 M borate buffer). Kinetics were followed by UV measurements of DNNO⁻ at 438 nm and equilibria by UV or NMR titration and nonlinear curve fitting with a stoichiometric 1:1 model.^b Rate constants (pseudo first order) at pH 10 = [OH⁻] = 10⁻⁴ M.^c Measured with completely ionized m-HB at pH = 12; K value corrected for salt concentration at pH 10.^d Obtained by numerical simulation of the ternary-complex kinetic with $K_6 = 10$ (250).^e k_4 and k_5 in s⁻¹ M⁻¹.

Chart I



nucleophilic substitution products X via Meisenheimer complexes (Chart I) to be a side process (see Experimental Section).

The reaction system (Scheme II) is comprised of 11 parameters; six of them were accessible by UV measurements and one by NMR measurements and one (K_8) is a function of three other equilibrium constants. Use of excess m-HB allowed the neglect of its concentration decrease. For the ternary-complex formation, we assumed a random mechanism (Scheme II).

The identification of the three parameters (K_6 , k_9 , and k_{10}) which cannot be determined by direct methods was then based on a simulation program, which allows for stepwise numerical integration of all reactions (Scheme II), for the description of equilibria which are formed much faster than those of the other reactions and therefore are not described by numerical integration, and for the least-squares fitting to experimentally observed time-

Table III. Activation Parameters^a

reaction	$\Delta S^\ddagger$	$\Delta H^\ddagger$	$\Delta G^\ddagger$
net	-14.9 ± 0.6	17.8 ± 1.4	18.4 ± 1.4
binary	-28.4 ± 0.8	12.8 ± 2.2	13.4 ± 2.2
cosubstrate	-28.1 ± 0.6	13.3 ± 1.4	13.9 ± 1.4
(β -naphthol			
cosubstrate	-32.7 ± 0.8	12.0 ± 2.2	12.6 ± 2.2
DHBS			
ternary	-33.5 ± 0.6	10.7 ± 1.4	11.3 ± 1.4
β -naphthol			
ternary	-39.3 ± 0.8	8.6 ± 2.2	9.3 ± 2.2
DHBS			

^a Determined from rates at 293, 298, 303, and 308 K at pH = 9.23 $\pm$ 0.05 with 0.05 M borate buffer in water with 1.2% acetonitrile; concentrations: [DNNA] = $(4.8 \pm 0.02) \times 10^{-5}$ M; [β -naphthol] = $4.95 \pm 0.01 \times 10^{-4}$ M; and [DHBS] = $(5.08 \pm 0.07) \times 10^{-4}$ M. Ionic strength kept constant at $I = 0.045$ by added NaCl; $\Delta H^\ddagger$ and $\Delta G^\ddagger$ (at 298 K) in kcal/mol, $\Delta S^\ddagger$ in cal/(K mol).

concentration curves on the basis of a modified SIMPLEX algorithm. The analysis of 19 kinetic measurements with molarities of $2.5 \times 10^{-5} < \text{[DNNA]} < 7.0 \times 10^{-5}$, $1.2 \times 10^{-3} < \text{[m-HB]} < 8.1 \times 10^{-3}$, and $3.5 \times 10^{-4} < \text{[CP66]} < 4.0 \times 10^{-3}$ reproduced the measured rate constants within the experimental error (Figure 2), with combinations of the unknown constants which range from $k_6 = 1 \text{ M}^{-1}$ (then $k_9 = 0.15 \text{ s}^{-1}$ and $k_{10} = 60 \text{ s}^{-1}$) to $K_6 = 10^3 \text{ M}^{-1}$ (then $k_9 = 0.2 \text{ s}^{-1}$ and $k_{10} = 0.087 \text{ s}^{-1}$). The applied, computerized, numerical curve-fitting method has the advantage over traditional linearization techniques of showing the whole range of parameters which satisfy the experimental data. From the broad minimum of square-error deviations obtained, we present in Table IV limiting constants which are selected on the assumption that the ternary complex should not be much more stable than the binary complex and that an acyl transfer with $k > 20 \text{ s}^{-1}$ is unlikely. Even with the rather high value of $K_6 = 250 \text{ M}^{-1}$, the effective concentration¹¹ amounts to $k_{\text{cat}}/k_0 = 500$. In order to illustrate the participation of the ternary-complex reaction, we indicate in Table IV also the relative contributions (initial rates) of different parallel reactions for

(11) Fersht, A. *Enzyme Structure and Mechanism*; W. H. Freeman: Reading, San Francisco, 1977; p 42.

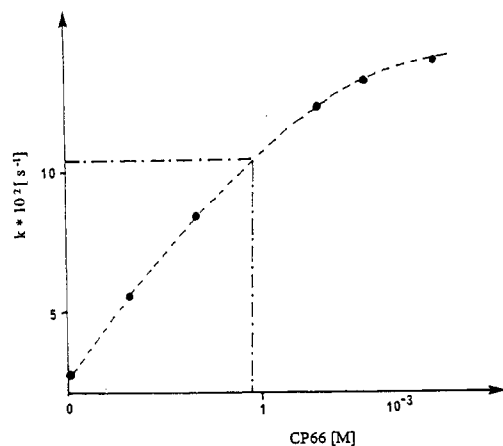


Figure 2. Acyl transfer from DNNA on *m*-HB with the ternary complex with CP66. Rate constants [10^{-2} s^{-1} , pseudo first order] vs [CP66] with [*m*-HB] = $8.07 \times 10^{-3} \text{ M}$; [DNNA] = $7.01 \times 10^{-5} \text{ M}$; other conditions, see Scheme II. Constants obtained from curve fitting; see Table I and text. The lines at [CP66] = $0.92 \times 10^{-3} \text{ M}$ depict the inhibition experiment with NS (see text).

selected concentrations.¹² At CP66 concentrations of 10^{-3} M , the transesterification catalyzed by the ternary complex dominates already over catalyzed and uncatalyzed hydrolysis and esterification.

Conclusions

Catalysis via ternary complexes is brought to high efficiency until now only in enzymes but can in principle be approximated

(12) Additional saturation curves with respect to varied cosubstrate concentrations could not be measured since at lower concentrations, the ternary complex contributes too little to a second-order process with cosubstrate alone (k_4 , Scheme II) and higher concentrations invariably lead to precipitation (e.g., with [CP66] = $2\text{--}10^2 \text{ mM}$ and [*m*-HB] = $0.3\text{--}1.3 \text{ mM}$).

Table IV. Selected Results from the Numerical Simulations^a

	$K_6 = 10 \text{ M}^{-1}$	$K_6 = 250 \text{ M}^{-1}$
k_9	14.6	0.635
k_{10}	6.1	0.27
k_{ter}	20.1	0.905
Contributions from the Ternary Complex ^b		
[CP66] = $2.0 \times 10^{-4} \text{ M}$	51%	48%
$1.0 \times 10^{-2} \text{ M}$	98%	97%

^a For explanations, see scheme II and text; $k_{\text{ter}} = k_9 + k_{10}$; all k values in s^{-1} ; $k_0 = k_1 = 2.36×10^{-3} . ^b Initial rate contributions, with [DNNA] = $7.01 \times 10^{-5} \text{ M}$ and [*m*-HB] = $8.07 \times 10^{-3} \text{ M}$.$

also in fairly simple synthetic host-guest entities. The complexation of *one* substrate *inside* the macromolecular cavity can be sufficient if the cosubstrate is bound *outside*, e.g., by electrostatic interactions. Much needs to be done before one can hope to obtain catalytic systems of practical value, but particularly ternary systems should allow reactions between two different compounds, interesting even from a synthetic point of view. Their structures can vary within a relatively large range with the shown combination of intra- and extracavity binding, the latter being less critical with respect of geometric fitting conditions.

A significant prerequisite for the rational development of biomimetic catalysis is the full characterization of the thermodynamic and kinetic parameters involved. We have shown that a combination of different measurement methods and numerical simulation procedures allows the analysis of complicated supramolecular catalytic systems which are not amenable to simplifications often possible with enzymes.

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