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The Aminolysis Of N-Hydroxysuccinimide Esters. A Structure-Reactivity Study

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for the hydrates of a series of aldehydes, β for hydrate ionization is -1.0 by definition. β_{13} is also expected analogously to be -1.0, and by definition β_{11} is 0. Based on literature values, particularly those of Sander and Jencks,[27] β_{14} is about -1.6; in this regard, the related addition of water to aldehydes and ketones exhibits a β of -1.4 (data of Table I in part). Accordingly, $\beta_{12} = -0.6$ (= -1.6 + 1.0). For R_1CHO and R_2CHO, whose hydrate pK_a differ by 5 units, log $K_u = -3.0$ (= 5.0 × -0.6). Thus, by using eq 4, $k^{R_1}_{mono}/k^{R_1}_{di} = 10^1$.

Supplementary Material Available: Part I estimates the pK values of hyponitrite species and hyponitrite-carbonyl and $-CO_2$ adducts; Part II estimates K_{add} for addition of hyponitrite N to acetaldehyde and compares K_{add} with kinetic data; Schemes VIII and IX are included (10 pages). Ordering information is given on any current masthead page.

The Aminolysis of *N*-Hydroxysuccinimide Esters. A Structure-Reactivity Study[1]

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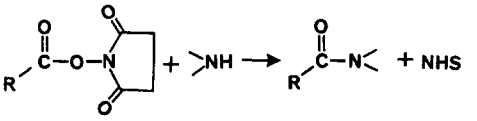
Abstract: Twelve amines, which vary substantially in basicity and in steric environment around N, have been allowed to compete—in anhydrous dioxane solution—in the aminolysis of the *N*-hydroxysuccinimide esters of unsubstituted, *p*-OCH_3, *p*-NO_2, and 3,5-$(NO_2)_2$ benzoic acids. The amines, which encompass a basicity range of 6.5 pK units, display a 10000-fold variation in reactivity in their reaction with the *p*-NO_2 ester. For the sterically unhindered amines, a Brønsted-type plot of log k_{obsd} vs. pK_a has a slope of ~0.7. The data fit a model (Satterthwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7018-7044) in which reversible formation of a tetrahedral intermediate is followed by rate-determining breakdown to products. Appreciable sensitivity to steric factors, as evidenced from the depressed rates with α-methylbenzylamine and diethylamine, substantiates reversible formation of a crowded tetrahedral intermediate prior to the rate-determining step. The Hammett ρ values for the competitive acylation of aniline, α-methylbenzylamine, and benzylamine, by substituted *N*-succinimidyl benzoates, are 1.4, 1.2, and 1.1, respectively. These values reflect the selectivity expected for these amines, and the substantial accumulation of charge density at the acyl C in the formation of the tetrahedral intermediate. Individual rate constants for the aminolysis of *N*-succinimidyl *p*-methoxybenzoate by *n*-butylamine, and by piperidine, both show first-order and second-order terms in [amine]. The general-base catalysis term is suggestive of a path involving proton transfer in the rate-determining step.

Carboxylic acid esters of *N*-hydroxysuccinimide (NHS), 1-hydroxy-2,5-pyrrolidinedione, have proved useful as intermediates in the synthesis of natural products and their analogues,[2] particularly affinity labels for cell receptors.[3] Additionally, NHS esters have been used "in situ" to enhance the sensitivity of hormonal assays,[4] to permit the selective isolation of membrane components without prior purification of the membrane,[5] and to facilitate the analysis of specific interactions among intrinsic membrane components[6] as well as between cell receptors and their ligands.[7] The report that NHS esters preferentially acylate amino groups under mild reaction conditions[8] has provided the impetus for the versatile exploitation of these reactive esters to further an understanding of biological systems at the molecular level.

However, the mechanistic details of the nucleophilic displacement of NHS from its ester linkage (eq 1) have not, to our



(1)

knowledge, been reported. This information can help define the scope and limitations of using NHS esters as synthetic intermediates and as biochemical probes. In this paper, we report on a study of the aminolysis of NHS esters in an anhydrous aprotic solvent, 1,4-dioxane. Competitive reactions were conducted to investigate the relationship between structure and reactivity in this acyl-transfer reaction. The relative reactivities of several primary and secondary amines, differing substantially in basicity and steric requirements around the nucleophilic N, provide information complimentary to that obtained from experiments in which the reactivity of the NHS esters was modified by substituents in the acyl portion of the molecule. In addition, individual rate constants for the aminolysis of the NHS ester of *p*-methoxybenzoic acid with *n*-butylamine and with piperidine were determined. The linear free energy relationships and the kinetic parameters derived from these experiments allow us to propose a reasonable model for the nucleophilic displacement of NHS esters in aprotic solvents.

(1) Results relating to portions of this work have been presented at the Third International EUCHEM conference on Correlation Analysis in Organic and Biological Chemistry, Louvain-la-Neuve, Belgium, July 15-18, 1985; and at the 191st National Meeting of the American Chemical Society, New York, New York, April 13-18, 1986.

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Table I. Properties of Substituted Benzoic Acid Derivatives[a]

derivative	benzoyl-	*p*-methoxy benzoyl-	*p*-nitro benzoyl-	3,5-dinitro benzoyl-
NHS	137–138	140–143	213.5–215	223–224.5
	(135[d])	(141–142[d])		
anilide	162.5–163.5[b]	166–169[b]	216–217[b]	238.5–241[b]
	162–163[c]	164.5–167.5[c]	213–215[c]	236–239[c]
	(162[e])	(168–171[f])	(211[e])	(234[f])
anisidide	157–158[b]	194–197[b]	199.5–201[b]	
	156.5–157[c]	193–194.5[c]	195–198[c]	
	(154[f])		(197[f])	
benzylamide	101.5–103.5[b]	127–129[b]	141.5–143[b]	185–186[b]
	101–104[c]	131.5–132[c]	139–139.5[c]	178–182[c]
	(104–107[f])	(131, 126[f])	(141–143[f])	
p-nitrobenzylamide			218–220.5[b]	
p-methylbenzylamide	139–140[b]	153.5–154.5[b]	157–158[b]	185–186[b]
	139–140[c]	154–156.5[c]	156–158[c]	
	(137[f])			
p-methoxybenzylamide		127–129[b]	137–138.5[b]	165–166[b]
		128–130[c]	130–131[c]	
α-methylbenzylamide	122–124[b]	139–141[b]	113–114.5[b]	172–174[b]
	119–121[c]	140–141[c]		
	(124[g])	(145[g])	(115–116[g])	
phenethylamide	114.5–115.5[b]	127.5–128.5[b]	148–149.5[b]	146–148.5[b]
	113–115[c]	126–129[c]	137.5–140.5[c]	
	(118–120[h])	(123–125[h])	(144–145[h])	
p-methoxyphenethylamide			149.5–150[b]	155–157[c]
			(147–147.5[i])	
n-butylamide		53–55[c]	103–105[b]	104.5–105.5[c]
		(45[f])	(102.5–103[f])	(105[k])
piperidide			120.5–121.5[b]	146–147[b]
			120–121[c]	
			(120.5[f])	(147[f])
diethylamide			64–66[b]	90–91[b]
			(60[e])	(92[l])

[a] Melting points are reported uncorrected in °C (lit. mp °C are in parentheses). [b] Derivative synthesized from acid chloride plus amine. [c] Derivative synthesized from NHS ester plus amine. [d] Horner, L.; Jordan, M. *Liebigs Ann. Chem.* **1978**, *9*, 1518. [e] Nair, P. G.; Joshua, C. P. *Indian J. Chem.* **1975**, *13*, 35. [f] *Beilsteins Handbuch der Organischen Chemie*; Springer-Verlag: Berlin, Heidelberg, New York. [g] Nerdel, F.; Goetz, H.; Wendenburg, J. *Ann.* **1959**, *627*, 106. [h] Nagubandi, S.; Fodor, G. J. *Heterocycl. Chem.* **1980**, *17*, 1457. [i] Vinokurov, V. G.; Troitskaya, V. A. *Zh. Obshch. Khim.* **1961**, *31*, 2991; Chem. Abstr. 15473b. [j] Barnett, J. W.; O'Connor, C. J. *J. Chem. Soc., Perkin Trans. 2* **1973**, *10*, 1331. [k] Berger, J.; Sorensen, A. D. *Acta Chem. Scand.* **1966**, *20*, 2002; Chem. Abstr. 66, 52029y. [l] Crampton, M. R.; Khan, H. A. *J. Chem. Soc., Perkin Trans. 2* **1973**, *6*, 710.

Experimental Section

General Methods. ^1H NMR spectra were recorded on a Varian EM360 or a Varian XL-300[9] with $CDCl_3$ as solvent and Me_4Si as an internal standard. IR spectra were recorded on a Perkin-Elmer 727B infrared spectrophotometer. UV spectra were recorded on a Perkin-Elmer 552 UV–vis spectrophotometer.

TLC analysis was performed on reverse-phase plates, Whatman KC18, developed with $MeOH/H_2O$ (70:30) or on silica-coated plates, Sigma T-6395, developed with $CHCl_3$/MeOH (90:10). Amides and NHS esters were visualized under UV light. The NHS esters were further confirmed by the immediate development of a strong brown color when treated with hydroxylamine ferric chloride.[10]

HPLC separation was accomplished on a C18 reverse-phase column, Supelco LC-18 (25 cm × 6.4 mm). A flow rate of 2 mL/min was provided by a Wescan solvent delivery system. Samples (10 μ) were introduced with a Rheodyne 7010 injector, and detection was accomplished by measuring the absorbance at 254 nm with a duPont 820 precision photometer detector.

Reagents. 1,4-Dioxane and THF were Aldrich Gold Label (99+% and 99.9%, respectively) and were used as obtained for the competitive experiments. For the individual kinetic experiments, the dioxane was further purified according to literature methods.[11] Methylene chloride, methanol, and ethanol were Fisher A.C.S. certified grade. Acetonitrile was Aldrich HPLC grade. Water for HPLC analysis was distilled, deionized on a Corning ultrahigh purity demineralizing cartridge, and filtered through a 0.45-μm nylon membrane. Aldrich chloroform-*d*, 99.8% D (1% v/v Me_4Si), was used as solvent in the NMR experiments.

All amines, with the exception of anisidine, 4-nitrobenzylamine, DL-α-methylbenzylamine, and diethylamine, were from Aldrich (97–99% pure). Diethylamine and DL-α-methylbenzylamine were reagent grade (Sigma). The liquid amines were purified by vacuum distillation over KOH; only the middle fraction was used for the competitive experiments. 4-Nitrobenzylamine, obtained as the hydrochloride from Aldrich, was treated with KOH, and the free amine was extracted into ether. After drying over anhydrous $MgSO_4$ and removing the ether, the amine was distilled under vacuum over KOH. Ansidine (Eastman Kodak) was purified by recrystallization.

1,3-Dicyclohexylcarbodiimide (DCC), NHS, benzoic acid, *p*-anisic acid, benzoyl chloride, *p*-anisoyl chloride, and 4-nitrobenzoyl chloride were from Aldrich. 3,5-Dinitrobenzoyl chloride was from Eastman Kodak. 3,5-Dinitrobenzoic and 4-nitrobenzoic acids were from Matheson, Coleman, and Bell.

Synthesis of NHS Esters. NHS esters were synthesized following the method of Buzas et al.[12] utilizing either 1,4-dioxane or THF as the solvent. The esters of benzoic, anisic, and *p*-nitrobenzoic acids were crystallized from 2-propanol/water. The ester of 3,5-dinitrobenzoic acid was crystallized from ethyl acetate/petroleum ether. NHS esters were recrystallized to a constant melting point (Table I). Yields as determined by HPLC analysis were nearly quantitative. Yields of recrystallized esters were typically better than 60%.

Synthesis of Amide Standards. Standards were prepared from the respective acid chloride and amine following established literature methods. The average yield was approximately 60%.

Synthesis of Amides from NHS Esters. To a solution of the NHS ester in dioxane, a five- to ten-fold excess of the amine was added. The reaction mixture was allowed to stand at room temperature until TLC analysis showed no NHS ester remaining. Depending upon the reactants, the time required for completion of the reaction varied from less than 2 min to more than 60 days.

(9) The assistance of the Washington University High Resolution NMR Facility, funded in part through NIH Biomedical Research Support Shared Instrument Grant 1 S10 RR02004, and a gift from the Monsanto Company are gratefully acknowledged.

(10) *Dyeing Reagents for Thin Layer and Paper Chromatography*; E. Merck: Darmstadt, Germany, 1976; p 49.

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Table II. Relative Reactivities[a] of Amines in the Aminolysis of Substituted Benzoyl-*N*-hydroxysuccinimide Esters

amine	pK_a[b]	anisoyl-*N*-hydroxysuccinimide	*p*-nitrobenzoyl-*N*-hydroxysuccinimide	3,5-dinitrobenzoyl-*N*-hydroxysuccinimide
aniline	4.60[e]		$1.2 \times 10^{-4} \pm 0.8 \times 10^{-4}$	
anisidine	5.34[e]		$1.9 \times 10^{-3} \pm 0.4 \times 10^{-3}$	
benzylamine	9.34[d]	1.0	1.0	1.0
p-nitrobenzylamine	8.38[d]		$1.5 \times 10^{-1} \pm 0.3 \times 10^{-1}$	
p-methylbenzylamine	9.41[c]	2.0 ± 0.6	1.2 ± 0.3	1.4 ± 0.2
p-methoxybenzylamine	9.56[d]		1.3 ± 0.5	1.8 ± 0.3
α-methylbenzylamine	9.59[d]	$1.4 \times 10^{-1} \pm 0.3 \times 10^{-1}$	$8.9 \times 10^{-2} \pm 0.9 \times 10^{-2}$	$6.5 \times 10^{-2} \pm 1.8 \times 10^{-2}$
phenethylamine	9.83[e]		1.3 ± 0.08	1.3 ± 0.1
p-methoxyphenethylamine			2.2 ± 0.2	1.9 ± 0.1
n-butylamine	10.60[e]	9.4 ± 1.1	5.2 ± 1.1	3.8 ± 0.3
piperidine	11.10[f]		$7.1 \times 10^{-1} \pm 1.5 \times 10^{-1}$	1.2 ± 0.1
diethylamine	10.98[e]		$7.1 \times 10^{-3} \pm 2.8 \times 10^{-3}$	

[a] Relative reactivities are normalized to benzylamine. Values are reported with confidence limit of 95%. [b] pK_a values are those for the amines in water.[15] [c] Carothers, W. H.; Bickford, C. F.; Hurwitz, G. J. *J. Am. Chem. Soc.* **1927**, *49*, 2908–2914. [d] Litvinenko, L. M.; Dadali, V. A.; Volovin, A. M.; Titov, E. T. *Reakts. Sposobnost Org. Soedin., Tartu. Gos. Univ.* **1966**, *3*, 75–85; Chem. Abstr. 69, 5699u. [e] Smith, J. W. In *The Chemistry of the Amino Group*; Patai, S., Ed.; Interscience Publishers: London, 1968; Chapter 4. [f] *Dictionary of Organic Compounds*; Pollock, J. R. A., Stevens, R., Eds.; Oxford University Press: New York, 1965; Vol. 5.

Amides were recovered, after dilution of the dioxane solution with water, by extraction into CH_2Cl_2. The organic phase was washed consecutively with 5% NaOH and 5% HCl and dried over anhydrous $MgSO_4$. Removal of the solvent by rotary evaporation left the amide, which was recrystallized to a constant melting point from ethanol/water or methanol/water. Recovered yields were generally above 50%.

The products of the reaction of the NHS ester of *p*-nitrobenzoic acid with several amines under conditions similar to the above, $CDCl_3$ as solvent, were further verified by NMR experiments. Simultaneous monitoring of the shift in the *p*-nitroaryl protons, the NHS methylene protons, and the appearance of the NHS amine salt ($\tau \sim 7.5$ for the NH_3^+ protons) during the course of the reaction revealed no products other than the amide and the NHS amine salt.

Competitive Reaction Rates: Determination of Relative Reactivities. In general, two solutions were prepared in 1,4-dioxane: one containing the limiting reagent (initial concentration, 3×10^{-3} M) and the other containing several competing reagents in concentrations in excess of the limiting reagent. Most reactions were conducted with the concentrations of competing reagents in 50–100-fold excess. In the few experiments where one reagent was much more reactive than the others, the concentration of the most reactive reagent was only 10–20-fold excess. For following the reactions, three different methods were employed. All three methods gave results agreeing within the 95% confidence limits established by the alternate methods.

Method 1. To a stirred solution (Vortex mixer) of the limiting reagent, an equal volume of the solution of the competing reagents was added. After thorough mixing, an aliquot was withdrawn and quenched with 4 M HCl in dioxane. The amine HCl salts were removed by retention on a strongly acidic cation exchange resin, Dowex 50W-X8, as a slurry in dioxane. The mixture was filtered through a glass-frit, and the reaction products were analyzed by reverse-phase HPLC (H_2O/CH_3CN (65:35)).

Method 2. This protocol was similar to the above method, except that the removal of the amines prior to HPLC separation was not conducted. At a fixed interval, usually 3 min after mixing, the sample was introduced into the HPLC system, and separation was accomplished with a solvent system of aqueous acetate buffer(0.2 M, pH 4.8)/CH_3CN in a ratio of 65:35 for the derivatives of the unsubstituted, *p*-nitro-, and *p*-methoxybenzoic acids and a ratio of 60:40 for the derivatives of 3,5-dinitrobenzoic acid.

Method 3. This method allowed us to check if the results were skewed due to mixing technique.[13] A Durrum stopped-flow apparatus (mixing efficiency of 99.5% within 2 ms) was used; the mixed solutions were collected and analyzed as outlined in method 2.

Kinetics. Pseudo-first-order reaction conditions were maintained by having [amine]/[ester] > 70 in dioxane solution. Equal volumes of the ester (initial concentration, 1×10^{-4} M) and amine solutions, temperature equilibrated (24.6 °C), were mixed. The disappearance of anisoyl-*N*-hydroxysuccinimide during the course of the reaction was monitored by the decrease in absorbance at 270 nm where no interference from other reactants or products could be observed. For reactions with halflives of less than 10 min, stopped-flow techniques were utilized.[14] For slower reactions, conventional spectrophotometry was used. Reactions were followed for more than 8 halflives. The data were evaluated by an exponential least-squares treatment of at least 12 points. The rate constant obtained for each concentration of amine represents four or more independent runs.

Table III. Relative Reactivities[a] of Substituted Benzoyl-*N*-hydroxysuccinimide Esters in the Acylation of Amines

substituent	σ	aniline	α-methyl benzylamine	benzylamine
p-OCH_3	−0.27	0.25 ± 0.07	0.41 ± 0.17	0.48 ± 0.14
p-H	0	1.0	1.0	1.0
p-NO_2	0.78	7.0 ± 2.4	6.8 ± 1.9	5.0 ± 1.1
3,5-$(NO_2)_2$	1.42	62 ± 38	39 ± 18	35 ± 15

[a] Relative reactivities are normalized to benzoyl-*N*-hydroxysuccinimide. Values are reported with confidence limit of 95%.

Results

Comparison of the spectral data (IR, UV, and NMR) and the physical (Table I) and chromatographic properties (TLC and HPLC mobility) of the amides synthesized from NHS esters with those synthesized from the corresponding acid chloride confirmed that the products of the reaction of amines with NHS esters were the expected amides. Furthermore, the amide and NHS amine salt were the only products observed in NMR experiments designed to monitor reaction progress during the aminolysis of NHS esters. Evaluation of the HPLC data showed that, under the conditions of the competitive aminolysis experiments (>200-fold excess of amine), the reaction was more than 96% complete within 3 min for the more reactive amines (e.g., benzylamine).

The relative reactivities of several primary and secondary amines (ΔpK_a 6.5), normalized to benzylamine, in the aminolysis of NHS esters are listed in Table II. For the NHS ester of *p*-nitrobenzoic acid, reactivities range over 10 000-fold, from aniline (the least reactive) to *n*-butylamine (the most reactive). The relative nucleophilicities give a straight line correlation with basicity[15] for the primary amines (excluding α-methylbenzylamine) investigated in this study (coefficient of correlation = 0.992). The evaluation of the data obtained for the NHS esters of *p*-methoxybenzoic- and 3,5-dinitrobenzoic acids is less satisfactory because of the limited number of amines (ΔpK_a 1.3) used. The most extensive data set was collected for the aminolysis of *p*-nitrobenzoyl-*N*-hydroxysuccinimide, and the interpretation of this data set will receive primary emphasis.

The relative reactivities of the NHS esters of substituted benzoic acids in the acylation of amines are presented in Table III. Three amines, which vary substantially in reactivity, were studied. Correlation of the relative reactivities with the Hammett substituent constants, σ, gives a slope, ρ, of 1.4 for aniline, 1.2 for

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(15) The pK_a values used are published values for the amines in water. Potentiometric determinations of base strength in aprotic solvents[16] have shown that relative basicity remains constant for either aqueous or nonaqueous solvents. Thus, while the intercept may vary, the slope of pK_a vs. log k/k_0 will remain constant.

Table IV. Observed Rate Constants[a] for the Aminolysis of Anisoyl-*N*-hydroxysuccinimide

[*n*-butylamine] (M)	k_{obsd} (s^{-1})	[piperidine] (M)	k_{obsd} (s^{-1})
0.015	5.06×10^{-3}	0.0147	6.32×10^{-5}
0.131	7.52×10^{-3}	0.0366	1.61×10^{-4}
0.255	2.12×10^{-2}	0.147	8.72×10^{-4}
0.272	1.98×10^{-2}	0.675	4.99×10^{-3}
0.510	5.24×10^{-2} [b]	0.920	1.06×10^{-2} [b]
0.540	6.17×10^{-2}	1.41	2.22×10^{-2}
0.910	1.49×10^{-1}		
0.970	1.55×10^{-1}		
1.21	2.52×10^{-1} [b]		
1.53	4.06×10^{-1}		

[a] Rates measured in 1,4-dioxane, T = 24.6 °C. [anisoyl-*N*-hydroxysuccinimide]$_0$ = 5.00×10^{-5}. [b] Relative standard deviation <7%, all other values <5%.

α-methylbenzylamine, and 1.1 for benzylamine (coefficient of correlation = 0.995, 0.999, and 0.996, respectively).

The values of k_{obsd} for the reaction of *n*-butylamine and piperidine with the NHS ester of *p*-methoxybenzoic acid are presented in Table IV. The observed rate constants fit a rate equation of the form

$$k_{obsd} = k_1[\text{amine}] + k_2[\text{amine}]^2$$

k_1 and k_2 were evaluated by a linear least-squares fit of k_{obsd}/[amine] vs. [amine]. The kinetic constants for the reaction of anisoyl-*N*-hydroxysuccinimide with *n*-butylamine were found to be $k_1 = 3.9 \times 10^{-2}$ s^{-1} and $k_2 = 1.4 \times 10^{-1}$ M^{-1} s^{-1} and for the analogous reaction with piperidine, $k_1 = 4.1 \times 10^{-3}$ s^{-1} and $k_2 = 7.8 \times 10^{-3}$ M^{-1} s^{-1}.

Discussion

The correlation of amine reactivity with basicity (Figure 1) yields a slope, β, of 0.73 for primary amines (excluding α-methylbenzylamine). As these experiments were not designed to separate the contribution of general-base catalysis, k_{gb}, from any other terms contributing to k_{obsd}, the slope is not a true Brønsted β as defined by β_{gb}. However, the rate constants for both a primary amine, *n*-butylamine, and a secondary amine, piperidine, do show k_{obsd} to consist of first-order and second-order terms in [amine]. Such terms have been shown[17] to yield nearly equivalent β values, β_{nuc} and β_{gb}, when evaluated by the Brønsted relationship. We, therefore, feel justified in considering the β value obtained in our experiments as a measure of charge development on the amino nitrogen at the transition state.

The magnitude of β indicates that the ability to stabilize charge development is quite important in the aminolysis reaction. This could have been interpreted in terms of extensive bond formation in the transition state for a rate-determining nucleophilic attack by the amine; however, such cases are known to exhibit a $\beta_{nuc} < 0.5$.[18] A value of $\beta_{nuc} \sim 0.7$ fits a model where the breakdown of a tetrahedral intermediate is rate-limiting. Faster rates, observed with increasing pK_a of the amine, may be the result of either an increased concentration of the intermediate or an enhancement of the transformation of the intermediate to products via general-base catalysis. The value of β is similar to that reported for the aminolysis of alkyl esters in aqueous systems[18] and has been deduced as being due to rate-determining breakdown of the tetrahedral intermediate.[18b,19] In those cases in which amine attack has been implicated as rate-determining, the β value is seen to drop to approximately 0.3, indicating that the transition state

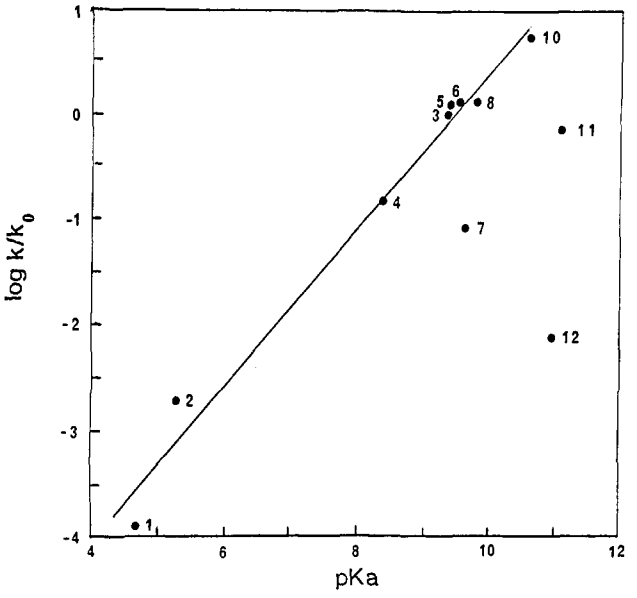

Figure 1. Relative reactivities (reactivities are normalized to benzylamine, i.e., reactivity of benzylamine, k_0 = 1) of amines in the aminolysis of *p*-Nitrobenzoyl-*N*-hydroxysuccinimide. Legend: (amine, symbol) aniline, 1; anisidine, 2; benzylamine, 3; *p*-nitrobenzylamine, 4; *p*-methylbenzylamine, 5; *p*-methoxybenzylamine, 6; α-methylbenzylamine, 7; phenethylamine, 8; *p*-methoxyphenethylamine, 9; *n*-butylamine, 10; piperidine, 11; diethylamine, 12.

is less sensitive to charge development at the amino nitrogen. For nitrogen nucleophilic attack on oxygen acyl esters, this break in the Brønsted-type plot is seen only when the amine is 4–5 pK units more basic than the leaving group.

A notable deviation from the linear correlation of reactivity with basicity is seen with a α-methylbenzylamine. Although, its basicity is midway between *p*-methoxybenzylamine and β-phenethylamine, the reactivity is more than an order of magnitude less than either. An even greater degree of deviation is seen in the comparison of piperidine and diethylamine, where the latter is approximately 100 times less reactive than the former, despite the similarity in basicity. The common trait of these two examples is their steric requirements. α-Methylbenzylamine imposes a methyl group into an incipiently crowded tetrahedral arrangement, while the alkyl groups of diethylamine can assume many more spatially demanding configurations than those of piperidine.

The Hammett ρ values of 1.1–1.4 indicate appreciable negative charge development on the acyl group in, or prior to, the rate-determining step. In the event of rate-determining breakdown of the tetrahedral intermediate, the observed ρ is a composite value of the ρ values for the preequilibrium and rate-determining steps. The increase in ρ with decreasing amine reactivity[20] is understandable in terms of the Bell–Evans–Polanyi principal[21] or the Hammond postulate[22] and serves only as an indication that the mechanism of the reaction remains constant for the amines investigated in this work. Menger and Smith[23] reported a value of ρ comparable to ours for the aminolysis of substituted phenyl benzoates in aprotic solvents when reactivity was evaluated as a function of substituents on the acyl portion of the molecule. They provide convincing evidence that the expulsion of the negatively charged leaving group (substituted phenoxides) is rate-determining. Their reported values of $\rho \sim 6$ for substituent effects in the leaving group points to a substantial development of negative charge and a pronounced ability of the leaving group to stabilize that negative

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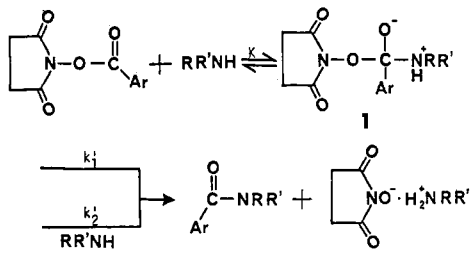
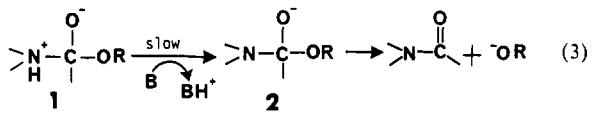
charge. Although we have no analogous data to support this argument, we favor the breakdown of the intermediate as the rate-determining step in view of the previous discussion regarding the value of β_{nuc}.

The rate expressions for both primary and secondary amines are consistent with reversible formation of a zwitterionic intermediate (**1**, eq 2) followed by rate-determining breakdown to products by either of two concurrent pathways, a general-base catalyzed, k_2', and a noncatalyzed, k_1', expulsion of NHS from the intermediate.



(2)

Work involving ^{18}O and secondary β-deuterium kinetic isotope effects[24] suggests that the transition state for noncatalyzed aminolysis involves acyl–O bond cleavage as the rate-determining step. The term representing general-base catalysis requires proton transfer in the rate-determining step. Persuasive evidence has been presented[19,24b] that proton transfer from the tetrahedral intermediate (**1**, eq 3) to the general-base catalyst, B, is rate-limiting for alkyl esters, followed by fast breakdown of the anionic intermediate **2** to products.



(3)

From the similarity in the values of β_{gb} and β values derived as a function of the pK_a of the leaving group, β_{lg}, for both alkyl and phenyl esters, it has been proposed[19] that the model of rate-limiting proton transfer is also valid for phenyl esters. Recent work,[25] however, suggests that the transition state for general-base-catalyzed decomposition of the tetrahedral intermediate of phenyl esters involves little tetrahedral character. Rather, the transition state is more nearly trigonal in nature, and thus proton transfer with the generation of a discrete intermediate cannot be rate-limiting. The greater steric requirement of phenyl vs. alkyl esters in a tetrahedral conformation has been proposed as the determinant for the change in the nature of rate-determining breakdown. If steric requirements are a decisive factor in the profile of the reaction coordinate, then NHS esters and phenyl esters may follow a similar path for this step.

The results presented in this paper, interpreted in the light of previous studies concerning the aminolysis of esters, are consistent with the view that this reaction proceeds through a tetrahedral intermediate in which the rate-determining step is the breakdown of the intermediate.

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Aromatic Ketone–Naphthalene Systems as Absolute Standards for the Triplet-Sensitized Formation of Singlet Oxygen, $O_2(^1\Delta_g)$, in Organic and Aqueous Media: A Time-Resolved Luminescence Study

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Abstract: Triplet states of simple aromatic hydrocarbons, naphthalene, biphenyl, and fluorene, have been formed with unit efficiency by triplet energy transfer from aromatic ketone triplets, themselves formed by pulsed laser excitation (355 nm) in aerated cyclohexane, dioxane, benzene, toluene, and acetonitrile. Time-resolved luminescence measurements, supported by 1,3-diphenylisobenzofuran bleaching experiments, have shown that in non-benzenoid environments the oxygen quenching of the aromatic hydrocarbon triplets gives singlet oxygen, $O_2(^1\Delta_g)$, with unit efficiency, i.e., $S_\Delta = 1.0$. In benzene and toluene this is not the case. An oxygenated benzophenone/naphthalene/micelle/water-d_2 system has been developed which produces naphthalene triplet and subsequently $O_2(^1\Delta_g)$ with unit efficiency. With appropriate controls this work has provided, for the first time, an accurate standard for the determination of $O_2(^1\Delta_g)$ quantum yields, $\phi_\Delta = \phi_T S_\Delta$, for water-soluble sensitizers which absorb at 355 nm. Naphthalene is the standard of choice in all media examined because its low triplet–triplet extinction coefficient at 355 nm maximizes the range over which the triplet yield is a linear function of the output energy of conventional Nd:YAG lasers with pulse widths in the 10–20-ns range. The establishment of naphthalene as a standard has confirmed that, under conditions producing identical $O_2(^1\Delta_g)$ yields, the emission intensity of this species is solvent dependent as a consequence of changes in the radiative rate constant, k_r.

1. Introduction

The principal mode of formation of singlet oxygen, $O_2(^1\Delta_g)$, in vivo and in vitro, involves electronic energy transfer from a sensitizer triplet-state to ground-state oxygen, $O_2(^3\Sigma_g^-)$. The quantitative aspects of such processes have been the subject of considerable discussion over the last two decades, and additional stimulus for research in this area has come from the recently developed technique of photodynamic therapy for the clinical

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