Aminolysis of Activated Esters of Indole-3-acetic Acid in Acetonitrile

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Seven activated esters of indole-3-acetic acid (IAA) [4-oxo-1,2,3-benzotriazin-3-yl indole-3-acetate (IAA-3HBO), succinimido indole-3-acetate (IAA-NHS), 5-norbornene-1,3-dicarboximido indole-3-acetate (IAA-NHND), phthalimido indole-3-acetate (IAA-NHP), naphthalimido indole-3-acetate (IAA-NHN), 4-nitrophenyl indole-3-acetate (IAA-4NP), 8-quinolyl indole-3-acetate (IAA-8HQ)] were prepared and characterized. Their kinetics of aminolysis with butylamine, piperidine, and pyrrolidine in acetonitrile were investigated. In general, the observed pseudo-first-order rate constants, k_{obs}, fit the two-term rate equation $k_{obs} = k_1[\text{amine}] + k_2[\text{amine}]^2$. For the aminolysis of six of the esters with piperidine there was no measurable k_2 term. The 8-hydroxyquinoline ester and *N*-hydroxynaphthalimide ester of indole-3-acetic acid had no measurable k_2 term for aminolysis with either pyrrolidine or piperidine. In general, the reactivity of the esters increased with increasing acidity of the conjugate acid of the nucleofuge and with increasing base strength of the nucleophilic amine. The IAA ester of NHP was observed to be ca. 100-fold more reactive than the IAA ester of 4NP although the pK_a's of the leaving groups are nearly the same. In all cases low activation energies were observed.

Introduction

The indole structure seems to be a template from which evolution has fashioned many biochemical receptors. It is well-known that indole-3-acetic acid (IAA) has highly developed stimulatory effects as a plant growth hormone in vascular plants and is a metabolic product of yeast and bacteria.[1] The widespread hormonal activity of IAA in the plant kingdom is paralleled by serotonin in the animal kingdom. In animals, serotonin (5-hydroxytryptamine, 5HT) has a multitude of functions,[2] the most notable of which is its role as a neurotransmitter. The specificity of serotonin receptors for the indole structure seems evident when one considers the acute psychological effects of the indole-based psychotropic alkaloid drugs, e.g., lysergic acid diethylamide (LSD), psylocybin, and psylocin.

Since many of the indole-specific receptors still elude characterization, it occurred to us that esters of IAA possessing adequate reactivity to react with amino residues at the receptor sites would offer enormous potential as affinity labels for indole-specific receptors. The so-called "activated esters",[3] frequently employed in protein synthesis, seemed to offer the required reactivity. Our interest in the potential of activated esters of IAA as convenient acylating agents, particularly as affinity labeling reagents in agriculture and pharmacology, prompted this study of the synthesis, characterization, and kinetics of aminolysis of several activated esters of IAA.

Whereas the previous studies in our laboratories focused only on NHS esters,[4,5] the present investigation was expanded to include a comparison of leaving-group abilities of several nucleofuges. Since it is generally accepted that the active sites of enzymes are buried in the hydrophobic regions of the enzyme, it has been suggested that aprotic solvents should offer a realistic medium for a mechanistic study.[6]

Results and Discussion

Preparation of Esters. Esters were prepared in THF at 0 °C following the method of Buzas et al.[7] by condensation of IAA with the hydroxyl moiety using 1,3-dicyclohexylcarbodiimide (DCC). The hydroxyl compounds used for ester preparation were *N*-hydroxysuccinimide (NHS), *N*-hydroxy-5-norbornene-1,3-dicarboximide (NHND), *N*-hydroxyphthalimide (NHP), *N*-hydroxynaphthalimide (NHN), 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (3HBO), 4-nitrophenol (4NP), and 8-hydroxyquinoline (8HQ). In all cases the esters were solid compounds with melting points ranging from 88 to 197 °C. By comparison with substituted phenyl esters of IAA,[8] the esters prepared from the *N*-hydroxy compounds all had high carbonyl stretching frequencies in the IR (Table I). High carbonyl stretching frequencies have been reported for the acetate and benzoate esters of 1-hydroxybenzotriazole[9] and *O*-acylhydroxylamine derivatives.[10] The shift to a higher frequency may, in general, be attributed to the greater electron-withdrawing inductive effect, hence higher acidity, of the *N*-hydroxy compounds compared to the substituted phenols.

Aminolysis of IAA Esters in Acetonitrile. The rates of aminolysis of the activated esters of IAA were conducted under pseudo-first-order conditions with amine concen-

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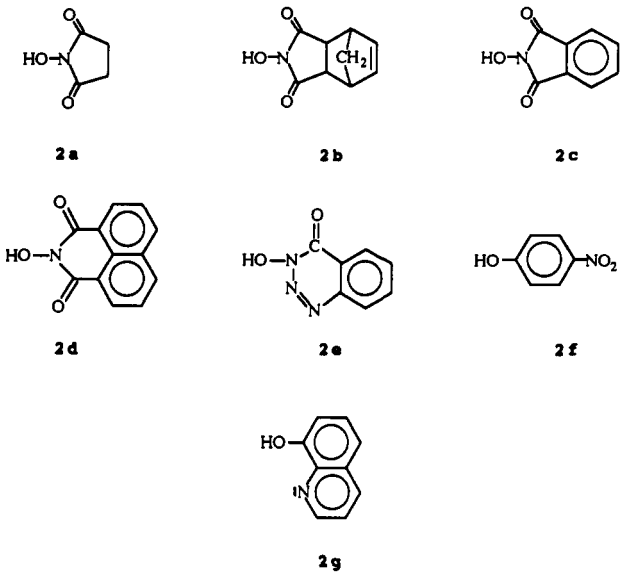
Chart I. Structures of Hydroxy Leaving Groups



2a 2b 2c 2d 2e 2f 2g

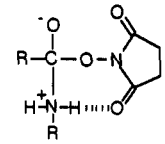
trations ranging from 20-fold to over 500-fold excess, with respect to ester concentration. Pseudo-first-order rate constants were measured for the reactions of the esters with butylamine, piperidine, and pyrrolidine at 15 °C, 25 °C, and 35 °C for most esters.

The general rate law in the present study is given by eq 1

$$k_{obs} = k_1[\text{amine}] + k_2[\text{amine}]^2 \quad (1)$$

where k_1 is the nucleophilic rate constant and k_2 is the catalytic (general-base) rate constant. Values for k_1 and k_2 (Table II) were calculated from the intercept and slope, respectively, of plots of k_{obs}/[amine] vs [amine]. Aminolysis of the IAA esters with butylamine ($BuNH_2$) show a slight downward concavity in plots of k_{obs}/[$BuNH_2$] vs [$BuNH_2$] (Figure 1). Greater deviation from eq 1 can be seen for the esters of NHP and 4NP. Similar leveling of reactivity with increasing base concentration has been reported for the reaction of substituted pyridines with 4-nitrophenyl phosphates and 4-nitrophenyl acetates.[11] These negative deviations from linearity were attributed to self-aggregation of the pyridines in aqueous solution to form unreactive dimers. Coetzee and co-workers[12] reported the formation of dimers and higher aggregates of amines in acetonitrile and showed that the formation constants increase with increasing amine concentration. In most cases in this study, however, only a slight deviation from linearity was observed, and k_2 was calculated from the slope of the best-fit line from plots of k_{obs}/[amine] vs [amine].

There was no observable k_2 term for aminolysis with piperidine, except in the case of IAA-3HBO. Aminolysis of the esters of 8HQ and NHP with piperidine and pyrrolidine showed no observable k_2 term. Evidence has been presented that strongly suggests intramolecular general base catalysis for the aminolysis of esters of 8HQ.[13] This would explain the absence of the k_2 term for aminolysis of this ester. By analogy, it has also been proposed that the exceptional reactivity of esters of NHS with amine nucleophiles may involve anchimeric assistance by formation of a hydrogen-bonded intermediate, 4.[3] The catalytic ability of crown ethers[14] and glymes[15] in ester aminolysis has been attributed to stabilization of the zwitterionic tetrahedral intermediate through formation of a hydrogen-bonded complex. In this study, however, the second-order dependence of the rates on amine concentration rules out intramolecular general base catalysis as the only catalyzed route, particularly since a hydrogen-bonded intermediate such as 4 seems viable.



4

The 1-hydroxybenzotriazole ester of benzoic acid was found to be 1000-fold more reactive than the corresponding 4-nitrophenyl ester, although the pK_a's of the leaving groups were reported to be nearly the same.[9] However, the similarity in the pK_a values was based on a pK_a of 7.39 for 1-hydroxybenzotriazole which was calculated from the tautomerization equilibrium data reported by Boyle and Jones.[16] Based on the pK_a of 4.3 reported by König and Geiger[17] one would expect the 1000-fold difference in reactivities. In fact, plots of log k vs pK_a of the leaving group, using published data,[18] indicate that *N*-hydroxy leaving groups correlate well with phenoxide leaving groups in aqueous media when the nucleophiles are amines. However, for similar plots using anionic nucleophiles,[18] positive deviations, corresponding to a 10–20-fold rate difference, of the *N*-hydroxy compounds from the Brönsted line are observed. In the present study, esters of NHS (pK_a 6.0)[19] and NHP (pK_a 7.0)[20] are approximately 100-fold more reactive than esters of 4NP (pK_a 7.15)[21] in aminolysis with butylamine and piperidine in acetonitrile.

The nucleophilicity of a nucleophile and the leaving ability of a nucleofuge often correlate well with the pK_a of the nucleophile or nucleofuge. The reactivity of activated esters of IAA increases with increasing base strength of the nucleophilic amine (Figure 2) and with increasing acidity of the leaving-group (Table II). However, we observed a disproportionate increase in reactivity when comparing the relative rates of aminolysis (acetonitrile) to the relative acidities (aqueous) of the leaving groups for esters of NHP and 4NP. Since the difference in reactivity of NHP and 4NP might be explained by a greater differentiation of acid strengths in acetonitrile we determined the equilibrium acidity constants for NHS (400 L mol^{-1}), NHP (798 L mol^{-1}), NHND (234 L mol^{-1}), and 4NP (328 L mol^{-1}) in acetonitrile spectrophotomet-

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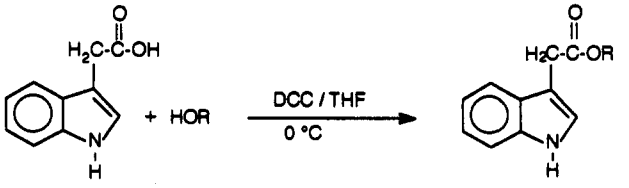
Table I. Properties of Activated Esters of Indole-3-acetic Acid



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HOR	structure[a]	CO (cm^{-1})	pK_a	analytical wavelength (nm)	[ester] (M)[b]
N-hydroxysuccinimide	**2a**	1824	6.0[c]	285	5×10^{-4}
N-hydroxy-5-norbornene 1,3-dicarboxmide	**2b**	1814	–[d]	285	5×10^{-4}
N-hydroxyphthalimide	**2c**	1809	7.0[e]	416	1×10^{-3}
N-hydroxynaphthalimide	**2d**	1815	8.25[e]	410	5×10^{-4}
3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one	**2e**	1803	4.3[f]	428	5×10^{-5}
4-nitrophenol	**2f**	1772	7.15[g]	314	5×10^{-4}
8-hydroxyquinoline	**2g**	1763	9.8[g]	339	5×10^{-5}

[a] See Chart I for structures of hydroxy leaving groups. [b] Concentration of ester used in aminolyses. [c] Reference 19. [d] pK_a not reported. [e] Reference 20. [f] Reference 17. [g] Reference 21.

Table II. Rate Constants[a] for the Aminolysis of Activated Esters of Indole-3-acetic Acid in Acetonitrile at 25 °C

ester		butylamine	piperidine[b]	pyrrolidine
IAA-NHS	k_1	4.95 ± 0.04[c]	7.25 ● 0.13	13.88 ● 0.06
	k_2	5.62 ± 0.21		4.01 ● 0.25
IAA-NHND	k_1	4.32 ± 0.02	6.56 ± 0.07	13.0 ± 0.4
	k_2	3.51 ± 0.12		4.6 ± 1.8
IAA-NHP	k_1	4.90 ● 0.11	5.35 ± 0.04	12.81 ± 0.07
	k_2	10.55 ± 0.63		15.35 ● 0.38
IAA-4NP	k_1	0.329 ± 0.007	0.78 ● 0.02	12.37 ± 0.19
	k_2			10.1 ● 0.9
IAA-NHN	k_1	–[d]	0.496 ± 0.008	0.865 ± 0.004
	k_2			
IAA-8HQ	k_1	–[d]	0.143 ± 0.002	0.114 ± 0.002
	k_2			
IAA-3HBO	k_1	1002 ± 11	3058 ● 171	2484 ● 73
	k_2	4026 ● 971	36834 ± 15504	19119 ± 6503

[a] Rate constants refer to eq 1. Units for k_1 and k_2 are $M^{-1}\ s^{-1}$ and $M^{-2}\ s^{-1}$, respectively. [b] No k_2 term was observed except for IAA-3HBO. [c] Standard deviation. [d] No kinetic study conducted.

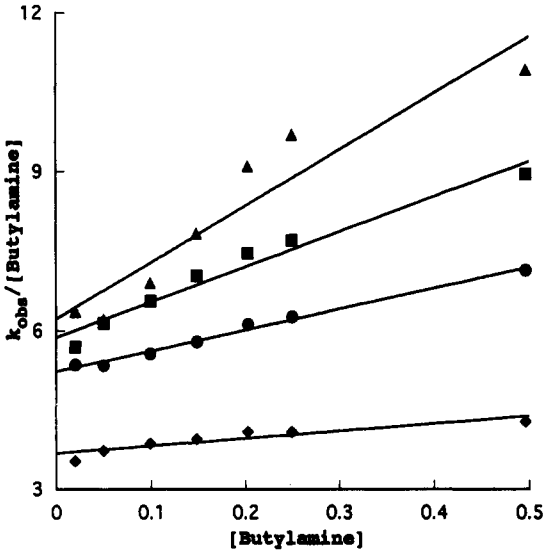

Figure 1. Plot of k_{obs}/[butylamine] vs [butylamine] for the aminolysis of activated esters of indole-3-acetic acid in acetonitrile at 35 °C: (■) IAA-NHS, (●) IAA-NHND, (▲) IAA-NHP, (◆) IAA-4NP [(k_{obs}/[butylamine]) × 10].

rically. These values revealed a slight leveling of acidities for NHS and NHP. This leveling of acidities is reflected in the similar reactivities of the esters of NHS, NHND, and NHP. However, a slight increase in the acidity ratio

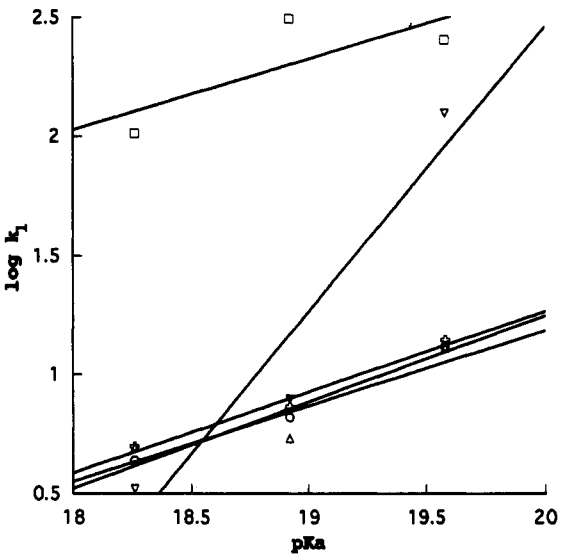

Figure 2. Plot of log k_1 vs pK_a (ref 12b) of the amine for aminolysis of activated esters of indole-3-acetic acid in acetonitrile at 25 °C: (□) IAA-3HBO [plotted values are log (k_1/10)], (✣) IAA-NHS, (○) IAA-NHND, (△) IAA-NHP, (▽) IAA-4NP [plotted values are log (k_1 × 10)].

of NHP vs 4NP is observed as one shifts from water (K_{aNHP}/K_{a4NP} = 1.4) to acetonitrile (K_{aNHP}/K_{a4NP} = 2.4). This change in acidities is hardly enough to account for the ca. 100-fold difference in reactivities.

Perhaps the large difference in reactivities of NHS, NHND, and NHP compared to 4NP might be explained satisfactorily by the greater extent of structural and electronic rearrangement involved during formation of the anion of 4NP. The greater catalytic ability of the oximes, compared to carboxylic acids and phenols of the same acid strength, has been attributed to a lesser degree of structural rearrangement during ionization of the oximes.[22] It is generally accepted that the stability of the anion of 4NP is due to contributions from its resonance structures, **5a** and **5b**. In fact, recent studies of acyl transfer from 4-nitrophenyl acetate have shown a measurable ^{15}N isotope effect.[23] This isotope effect has been attributed to bonding

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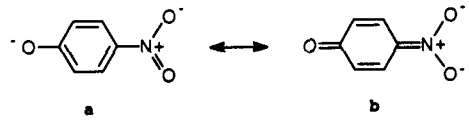
changes that occur on the nitro group as a result of the delocalization of charge on the incipient 4-nitrophenolate ion. Comparison of the resonance structures **5a** and **5b** with the resonance structures of NHP, **6a** and **6b**, serves to illustrate the greater degree of electronic rearrangement involved in the ionization of 4NP.



a b

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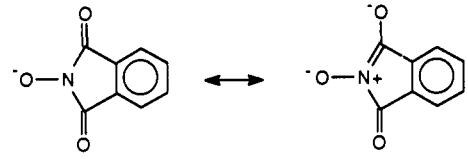

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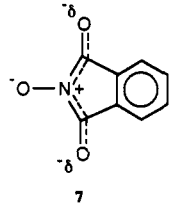
6

X-ray crystallographic analysis of esters of NHP indicate that the hydroxyl O, the imide N, and the two carbonyl C's of NHP are coplanar.[24] This planar structure requires the nitrogen to be sp^2 hybridized and the lone electron pair to be in a p-orbital perpendicular to the plane defined by the nitrogen atom and its three substituents. Delocalization of the lone electron pair into the neighboring electron-deficient carbonyl groups would leave a partial positive charge on the nitrogen. The overlap of the nitrogen p-orbital with the p-orbitals of the adjacent acyl carbons would impart considerable double bond character to the C(O)–N bonds. The extended conjugation of the two carbonyl groups and the nitrogen may be more accurately depicted as in structure **7**. The increasing electron density on the oxygen of the incipient anion of NHP can be stabilized by the partial positive charge on the neighboring nitrogen accompanied by little structural or electronic rearrangement. The extent of double bond character of the C(O)–N bond in cyclic imides has been demonstrated by the ability of N-substituted phthalimides[25] and N-substituted 2,3-naphthalimides[26] to undergo $2\pi + 2\pi$ cycloadditions with olefins to produce benzazepinediones and naphthazepinediones, respectively.



7

We subjected the IAA ester of NHS to aminolysis with butylamine-*N*-d_2 ($BuND_2$) [seven concentrations ranging from 2.41×10^{-2} to 6.0×10^{-1} M] at 25 °C. The results were compared to aminolysis with $BuNH_2$ over the same range of amine concentrations since curvature in the plots of $k_{obs}/[BuNH_2]$ vs $[BuNH_2]$ makes the calculated rate constants somewhat concentration dependent. Second-order ($5.25\ M^{-1}\ s^{-1}$ for $BuND_2$; $4.84\ M^{-1}\ s^{-1}$ for $BuNH_2$) and third-order ($4.33\ M^{-2}\ s^{-1}$ for $BuND_2$; $5.08\ M^{-2}\ s^{-1}$ for $BuNH_2$) rate constants were calculated by proportionally weighted least squares analysis of plots of $k_{obs}/[amine]$ vs [amine]. From these rate constants we obtained values of 0.92 for k_{1H}/k_{1D} and 1.2 for k_{2H}/k_{2D}. A value less than one for k_{1H}/k_{1D} suggests enhanced nucleophilicity of the deuterated butylamine. The apparent increase in nucleophilicity of butylamine upon deuteration is to be expected since the additional mass of the deuterium atoms should decrease the N–H(D) stretching frequency resulting in an increase in electronegativity of the nitrogen. The increased electronegativity of the nitrogen would further repel the lone electron pair making them more available to a promixate electrophile. Menger and Smith[27] pointed out that such small isotope effects are consistent with Swain's "solvation rule"[28] which states that no primary isotope effect will be observed for proton transfer between heteroatoms in reactions involving bond changes on a carbon in the rate-determining step.

Table III. Activation Energies[a] for the Aminolysis of Activated Esters of Indole-3-acetic Acid in Acetonitrile

ester		*n*-butylamine	piperidine[b]	pyrrolidine
IAA-NHS	k_1	2.9 ± 0.02	4.3 ± 0.3	4.2 ± 0.1
	k_2	3.5 ± 0.3		−5.4 ± 3.4
IAA-NHND	k_1	3.0 ± 0.3	4.1 ● 0.4	–[c]
	k_2	2.9 ± 0.5		
IAA-NHP	k_1	2.7 ± 0.6	4.3 ● 0.04	4.5 ± 0.3
	k_2	3.6 ± 1.2		−0.2 ± 1.1
IAA-NHN[b]	k_1	–[c]	5.7 ± 0.4	–[c]
IAA-4NP	k_1	6.7 ± 2.6	4.9 ± 0.5	3.7 ± 0.06
	k_2	−1.2[d]		0.93 ± 0.03
IAA-8HQ[b]	k_1	–[c]	5.4 ± 0.4	6.2 ± 0.3
IAA-3HBO	k_1	3.1 ± 0.1	–[c]	–[c]
	k_2	−18 ± 7		

[a] Activation energies are in kcal/mol. [b] No observable k_2 term. [c] Temperature study not conducted. [d] Calculated using k_2 values at 15 °C and 35 °C.

Temperature Dependence. Aminolyses of selected esters of IAA were studied at 15 °C, 25 °C, and 35 °C. The concentrations of amine solutions were corrected for determinations at temperatures other than 25 °C using eq 2, where the coefficient of thermal expansion was determined from densities of acetonitrile given by Timmermans.[29]

$$\ln (C/C_o) = -1.35 \times 10^{-3} (T - T_o) \qquad (2)$$

C_o is the concentration at T_o, i.e., the concentration of the amine prepared at 25 °C; and C is the concentration of amine at the new temperature.

Activation energies were calculated from the Arrhenius equation by constructing plots of ln k_1 vs $1/T$(K) and ln k_2 vs $1/T$(K) and are summarized in Table III. Activation energies calculated from the nucleophilic rate constants, k_1, were in the range of 2.65–6.73 kcal mol^{-1}. Activation energies calculated from the general base rate constants, k_2, were also low and in some cases negative. These activation energies are low by comparison to those reported for the aminolysis of substituted phenyl acetates in

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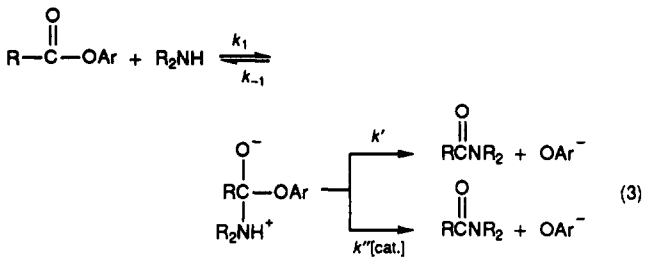
water.[18,30] Low activation energies have been reported for the butylaminolysis of NHS esters of substituted phenylacetic acids in acetonitrile[5] and negative activation energies have been reported for the butylaminolysis of 4-nitrophenyl trifluoroacetate in aprotic solvents.[31] These low, and in some cases negative, activation energies have been attributed to an exothermic preequilibrium in which the reactants are in equilibrium with a tetrahedral intermediate, eq 3. Thus, the activation energy is expected



(3)

to decrease with increasing base strength of the nucleophilic amine, as well as with increasing Lewis acid strength of the carbonyl carbon. For the hydrazinolysis of substituted phenyl acetates,[30] $\Delta H^{\ddagger}$ has been shown to increase as the substituent becomes less electron attracting. Aminolysis of substituted and unsubstituted phenyl acetates with ethylamine, morpholine, and piperidine[18] showed a similar correlation between $\Delta H^{\ddagger}$ and the acidity of the leaving group, while there was no obvious correlation between $\Delta H^{\ddagger}$ and base strength of the nucleophilic amine. There are no consistent trends in our data that would suggest any correlation of thermodynamic parameters with nucleophilicity or leaving group ability.

Experimental Section

General Methods. ^{1}H NMR spectra were recorded on a Varian VXR 200 or a Varian EM360 with CD_3CN as solvent and Me_4Si as an internal standard. IR spectra were recorded on a Perkin-Elmer 1750 Fourier transform infrared spectrophotometer. UV spectra were recorded on a Perkin-Elmer 552 UV–vis spectrophotometer. TLC analysis was performed on Whatman K6F 250-μm plates, developed with chloroform/benzene/methanol (58:40:2) and visualized under short-wave (254-nm) UV light.

Reagents. Acetonitrile, methanol, chloroform, and tetrahydrofuran were Aldrich HPLC grade and were used as received unless stated otherwise. Methylene chloride was from Fisher. *N*-Hydroxysuccinimide (NHS), *N*-hydroxynaphthalimide sodium salt (NHN-Na), *N*-hydroxy-5-norbornene-2,3-dicarboximide (NHND), 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (3HBO), and 1,3-dicyclohexylcarbodiimide (DCC) were from Aldrich. Indole-3-acetic acid (IAA) and *N*-hydroxyphthalimide (NHP) were from either Aldrich or Fluka. 4-Nitrophenol (4NP) was from Eastman. 8-Hydroxyquinoline (8HQ) was from Riedel-De HAEN AG. *N*-Hydroxynaphthalimide (NHN) was prepared from the sodium salt by acidifying an aqueous solution of the salt with hydrochloric acid followed by recrystallization from 2-propanol. Deuterium oxide was from either MSD Isotopes or Cambridge Isotope Laboratories, 99.8 atom % D and 99.9 atom % D, respectively. Water (H_2O) was first distilled from potassium permanganate and then redistilled from an all-glass still.

Amines. Butylamine, 99%, piperidine, 98%, pyrrolidine, 99%, and triethylamine, 99%, were from Aldrich and were fractionally distilled over CaH_2 prior to use. Only the center one-third from the distillates was used in kinetic studies. Triethylamine was used as received from Aldrich. N-Deuterated butylamine was prepared as described by Menger and Smith.[27] Evidence for deuteration of the amine was the disappearance of the NH_2 resonance in the ^{1}H NMR spectrum.

Preparation of Amine Solutions: Method 1. Preparation by Weight. Solutions of amines were prepared by weight in a thermostated water bath at 25.00 ± 0.05 °C. The amines were weighed and transferred into volumetric flasks that were filled to approximately 75% capacity with acetonitrile. The solutions were thermally equilibrated for 20 min after which they were slowly brought to volume by addition of acetonitrile which had been thermostated in the same water bath.

Method 2. Preparation by Volumetric Dilution. The concentrations of the amine solutions for the aminolysis of IAA-3HBO were too dilute to allow accurate preparation by weight. These solutions were prepared by volumetric dilution of a stock solution which had been prepared as described in method 1.

Beer's Law Adherence. Rate constants were measured by observing the increase in absorbance at a wavelength characteristic of the anion of the leaving group. The wavelength exhibiting the greatest net change in absorbance was used as the analytic wavelength. Adherence to Beer's Law at the analytic wavelength was checked by constructing plots of absorbance versus concentration. In all cases the absorbing species was the anion of the leaving group. Solutions of the leaving groups were prepared in acetonitrile with an excess of triethylamine to promote ionization. Typical concentrations of the leaving groups ranged from 10^{-5} to 10^{-3} M while the concentration of triethylamine was fixed at 9×10^{-2} M. Negative deviations from Beer's Law were observed at concentrations as low as 10^{-4} M. The concentrations of the samples used in the kinetic runs were chosen so as to be on the linear portion of the plot.

Kinetic Methods. The rates of aminolysis of the esters of indole-3-acetic acid, in most cases, were too fast to be measured by conventional means and required the use of stopped-flow techniques. Rate data were obtained using a Durrum–Gibson stopped-flow instrument equipped with a Durrum Photometric Log Amplifier, Model D-131, and connected to a Biomation Transient Recorder, Model 802. The latter was interfaced to an Apple IIe computer via a Metrabyte APM-08 Data Aquisition and Control board and the associated STA-AP terminal connector box. The Apple IIe computer was equipped with the necessary software for data collection, reduction, and presentation. Changes in absorbance with respect to time were recorded for each determination. Proportionally weighted least-squares analysis was performed on each set of data. The observed rate constant (k_{obs}) was taken to be the negative of the slope of the plot of ln $\{(A_\infty - A_t)/(A_\infty - A_0)\}$ vs time, where A_∞ is the absorbance at 10 or more half-lives, A_0 is the absorbance at time $t = 0$, and A_t is the absorbance at any time between A_0 and A_∞. Aminolyses were conducted in HPLC-grade acetonitrile, as received, since there appeared to be no advantage to further purification. Temperature control was achieved by means of a circulating water bath. A Haake thermoregulator was used to maintain water temperature at the desired temperature to within ±0.05 °C.

Pseudo-First-Order Adherence. As a test for adherence to a pseudo-first-order rate law, the observed rate constants, k_{obs}, were determined for two or more initial concentrations of the ester spanning at least a 10-fold change in concentration. When initial ester concentrations corresponded to the linear range of the Beer's Law plot, observed rate constants were the same within experimental error. Table I lists the concentrations and wavelengths used for individual esters for the kinetic studies.

Synthesis of Esters. Approximately equimolar amounts of indole-3-acetic acid and the hydroxy moiety were combined in the requisite amount of THF to ensure solubility at 0 °C. A 10% molar excess of DCC was dissolved in a volume of THF equal to that used for the IAA solution. After cooling both solutions to 0 °C in an ice bath they were combined and allowed to react at 0 °C. The reaction times varied from 8 to 24 h. The 1,3-dicyclohexylurea (DCU) that formed during the course of the reaction was removed by vacuum filtration. The volume of the filtrate was reduced by evaporation under diminished pressure. The remaining oily residue was taken up in a minimum of CH_2Cl_2 and filtered again to remove remaining DCU. The crude products, recovered by evaporating the CH_2Cl_2, were recrystallized from 2-propanol. Yields of recrystallized esters ranged from 22% to 72%.

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Succinimido indole-3-acetate (2a): mp 139–140 °C; IR (KBr) 3425 (NH), 1824 (CO) cm^{-1}; ^1H NMR (CH_3CN) δ 2.74 (s, 4 H), 4.09 (s, 2 H), 7.08–7.59 (m, 5 H, indole), 9.30 (br, NH). Anal. Calcd for $C_{14}H_{12}N_2O_4$: C, 61.76; H, 4.44; N, 10.29; O, 23.51. Found: C, 61.24; H, 4.42; N, 10.23; O, 23.27.

5-Norbornene-1,3-dicarboximido indole-3-acetate (2b): mp 158–159.5 °C; IR (KBr) 3394 (NH), 1814 (CO) cm^{-1}; ^1H NMR (CH_3CN) δ 1.55 and 1.64 (2 d, 2 H), 3.35 (s, 4 H), 4.03 (s, 2 H), 6.12 (s (br), 2 H), 7.03–7.55 (m, 5 H, indole), 9.27 (br, NH). Anal. Calcd for $C_{19}H_{16}N_2O_4$: C, 67.85; H, 4.79; N, 8.33; O, 19.03. Found: C, 68.06; H, 4.79; N, 8.59; O, 19.32.

Phthalimido indole-3-acetate (2c): mp 157–159 °C; IR (KBr) 3430 (NH), 1809 (CO) cm^{-1}; ^1H NMR (CH_3CN) δ 4.17 (s, 2 H), 7.06–7.63 (m, 5 H, indole), 7.86 (s, 4 H, arom), 9.31 (br, NH). Anal. Calcd for $C_{18}H_{12}N_2O_4$: C, 67.50; H, 3.78; N, 8.75; O, 19.98. Found: C, 67.51; H, 3.71; N, 8.77; O, 19.23.

Naphthalimido indole-3-acetate (2d): mp 197–198 °C; IR (KBr) 3385 (NH), 1815 (CO) cm^{-1}; ^1H NMR (CH_3CN) δ 4.23 (s, 2 H), 7.12–7.70 (m, 5 H, indole), 7.82–7.90 (m, 2 H, arom), 8.41 and 8.57 (2 d, 4 H, arom), 9.33 (br, NH).

4-Oxo-1,2,3-benzotriazin-3-yl indole-3-acetate (2e): mp 189–191 °C; IR (KBr) 3402 (NH), 1803 (CO) cm^{-1}; ^1H NMR (CH_3CN) δ 4.28 (s, 2 H), 7.08–7.68 (m, 5 H, indole), 7.88–8.35 (m, 4 H, arom) 9.35 (br, NH).

4-Nitrophenyl indole-3-acetate (2f): mp 88–89 °C; IR (KBr) 3435 (NH), 1772 (CO) cm^{-1}; ^1H NMR (CH_3CN) δ 4.08 (s, 2 H), 7.06–7.66 (m, 5 H, indole), 7.29 and 8.22 (2 d, 2 H, arom), 9.28 (br, NH).

8-Quinolyl indole-3-acetate (2g): mp 183–185 °C; IR (KBr) 1763 (CO) cm^{-1} (the diminished intensity of the indole NH absorbance, usually occurring at ca. 3400 cm^{-1}, and the broadening of the NH resonance in the ^1H NMR spectrum is indicative of hydrogen bonding with the nitrogen free electron pair of quinoline); ^1H NMR (CH_3CN) δ 4.22 (s, 2 H), 7.11–8.94 (m, 11 H, arom), 9.27 (br, NH).

Synthesis of Amides. The amides were prepared from the IAA-NHP ester and the corresponding amine. To 20 mL of CH_2Cl_2 were added 1 mmol of IAA-NHP and 1 mL of the amine.

1-Pyrrolidinylindole-3-acetamide. After allowing 5 min for reaction the solution was flooded with 100 mL of water. The volume of the solution was reduced to ca. 40 mL by evaporation under reduced pressure. The solutions were chilled to effect crystallization of the product which was collected on a Hirsch funnel and recrystallized from hexane. Yield of recrystallized product was 64%: mp 136–137 °C; IR (KBr) 1629 (CO) cm^{-1}; ^1H NMR (CH_3CN) δ 1.76–1.97 (m, 4 H), 3.35 and 3.50 (2 t, 4 H), 3.70 (s, 2 H), 6.99–7.60 (m, 5 H, indole), 9.22 (br, NH).

1-Piperidinylindole-3-acetamide. Procedure for synthesis is the same as described above for 1-pyrrolidinylindole-3-acetamide. Yield of recrystallized (hexane) product was 63%: mp 106–107 °C. IR (KBr) 1615 (CO) cm^{-1}; ^1H NMR (CH_3CN) δ 1.33–1.56 (m, 6 H), 3.48 (m, 4 H), 3.77 (s, 2 H), 6.99–7.60 (m, 5 H, indole), 9.21 (br, NH).

***N*-Butylindole-3-acetamide.** After allowing 5 min for reaction the solution was alternately washed, three times each, with 20-mL portions of 5% HCl and 5% $NaHCO_3$ solutions followed by three washings, 30 mL each, with water. The CH_2Cl_2 solution was dried over anhydrous Na_2SO_4. Removal of the solvent yielded a viscous brown oil which was chromatographed on silica gel using $CHCl_3$ and dried at 100 °C under reduced pressure. All attempts to effect crystallization of the amide were unsuccessful. Yield of recovered product was 83%: IR (KBr) 1648 (CO) cm^{-1}; ^1H NMR (CH_3CN) δ 0.84 (t, 3 H, CH_3), 1.13–1.39 (m, $(CH_2)_2$), 3.10 (m, CH_2), 6.36 (br, 1 H), 7.00–7.55 (m, 5 H, indole), 7.59 (residual $CHCl_3$ from chromatography), 9.26 (br, NH).

Completeness of Reaction. Thin-layer chromatography (TLC) was used to test for completeness of reaction of the IAA esters and the amines. The amide products, used for references, were prepared as described above. In all cases the reactions were shown to be quantitative.

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Supplementary Material Available: Procedure for spectrophotometric determination of equilibrium acidity constants in acetonitrile, effect of added water on observed rate constants, tables of rate constants at 15 °C, 25 °C, and 35 °C, table comparing observed rate constants for aminolysis of IAA-NHS with $BuNH_2$ and $BuND_2$, and ^1H NMR spectra of compounds described in the Experimental Section (22 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.