Multiproton Catalysis in the Hydrolysis of 3,5-Dinitroaspirin[1,2]

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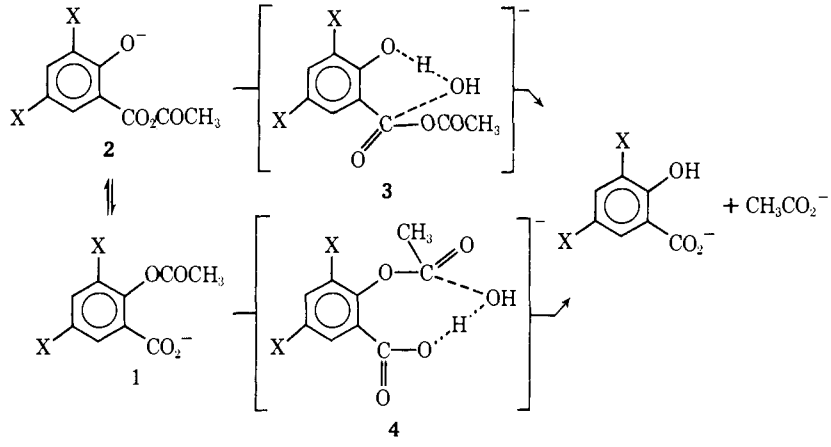
Abstract: The uncatalyzed hydrolysis of the anion of 3,5-dinitroaspirin, shown by Fersht and Kirby to proceed at least in part through an anhydride intermediate, exhibits rate constants k_W^n in mixtures of deuterium oxide (atom fraction n of deuterium) and protium oxide (atom fraction $1 - n$ of protium) which are described by the equation $10^4 k_W^n = 1.27[0.39(1 - n + 0.50n)(1 - n + 0.83n)^2 + 0.61(1 - n + 0.52n)]$ sec^{-1}. The intermediate salicylic acetic anhydride anion apparently hydrolyzes both at the salicyl carbonyl (39%, by classical anhydride hydrolysis) and at the acetyl carbonyl (61%, with protic bridging to the phenoxy center). The observed solvent isotope effect of 2.2 (k_H/k_D) is a weighted average of the effects for salicyl attack (2.9) and acetyl attack (1.9) and is coincidently equal to the effect for aspirin itself, which reacts with simple intramolecular protolytic catalysis by carboxylate.

In their now classic delineation of the dual mechanisms of intramolecular carboxylate catalysis of ester hydrolysis,[4] Fersht and Kirby showed that while O-to-O migration in all aspirin derivatives is rapid compared to hydrolysis, only in the case of highly stabilized intermediates such as that (**2**) from 3,5-dinitroaspirin anion (**1**, X = NO_2) is this nucleophilic pathway a feasible hydrolytic route. Less activated compounds such as aspirin itself (**1**, X = H) pass through the simple protolytic pathway (transition state **4**). Part of the evidence favoring **4** as the transition state in aspirin hydrolysis was a solvent isotope effect, k_{H_2O}/k_{D_2O} of 2.2, consistent with the protolytic catalysis depicted in the structure. We recently showed[5] this to be a case of "one-proton catalysis" with the bridging proton contributing the entire isotope effect and the hydroxyl proton (projecting to the right in **4**) and other protons contributing nothing. Somewhat surprisingly, Fersht and Kirby had found a very similar isotope effect (k_{H_2O}/k_{D_2O} = 2.1 at 39°) in the hydrolysis of 3,5-dinitroaspirin which clearly proceeds through **2**, as demonstrated by ^{18}O-labeling and methanol-trapping experiments. This suggested[4] a transition state like **3**, in which the aryl oxide center functioned as a general base catalyst. Further confirmatory evidence for **3** included $\Delta S^* = -22.6$ eu and the observation that methylation of the putatively catalytic oxygen led to a different product distribution. Actually, the *uncatalyzed* hydrolysis of anhydrides occurs with large, normal solvent isotope effects[6] and negative entropies of activation,[7] and the product change on methylation might arise from electrostatic-charge differences, so that establishment of structure **3** had to be considered tentative. We therefore considered it worthwhile to conduct a *proton inventory* (listing of the protons contributing to the solvent isotope effect and the contribution of each, as derived from rate measurements in mixtures of protium and deuterium oxides)[8] for the hydrolysis of 3,5-dinitroaspirin. Our findings are reported herein.



Results

Kinetics. The first-order rate constants of Table I verify the buffer catalysis observed by Fersht and Kirby[4] and are within 3% of their values when the latter are extrapolated to 25°, suggesting no change in mechanism with lowering of temperature. Values of k_W and k_B, obtained by linear least-squares fitting of

(1) Catalysis in Ester Cleavage. VI. For part V, see A. E. Williams, J. K. Lee, and R. L. Schowen, *J. Org. Chem.*, **38**, 4053 (1973).

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(3) Holder of a Research Career Development Award of the National Institute of General Medical Sciences, 1968–1973.

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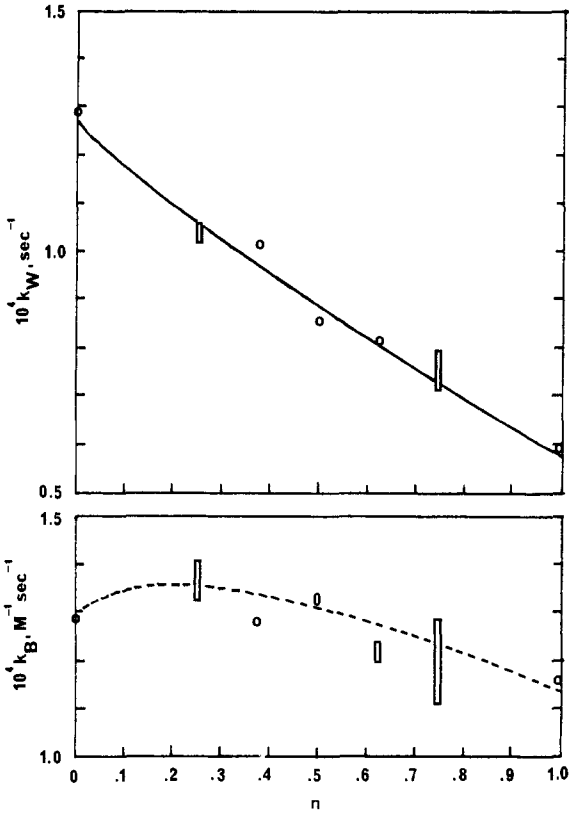


Figure 1. Dependences of k_W and k_B from eq 1 on the atom fraction of deuterium n in mixtures of protium and deuterium oxides. The dashed line in the lower part of the figure is arbitrarily drawn while the solid line in the upper part is calculated from eq 5b with $10^4 k_W^0 = 1.27$ sec^{-1}.

Table I. First-Order Rate Constants[a] ($10^5 k_{obsd}$, sec^{-1}) for Hydrolysis of 1.84×10^{-5} *M* *O*-Acetyl-3,5-dinitrosalicylic Acid in Acetate Buffers[b] ($[NaO_2CCH_3]/[HO_2CCH_3] = 10.02 \pm 0.22$) in Binary Mixtures of Protium and Deuterium Oxides (Atom Fraction of Deuterium n) at $25.00 \pm 0.01°$

	[NaO_2CCH_3], *M*			
n	0.1762	0.3522	0.5289	0.8046
0.000	15.17 ±0.17	17.59 ±0.07	19.79 ±0.35	22.02 ±0.89
0.250	12.73 ±0.09	15.15 ±0.07	17.88 ±0.27	19.86 ±0.10
0.375	12.42 ±0.26	14.69 ±0.04	17.03 ±0.23	19.17 ±0.08
0.500	10.92 ±0.29	13.11 ±0.11	15.56 ±0.35	17.93 ±0.11
0.625	10.26 ±0.01	12.55 ±0.06	14.76 ±0.06	16.69 ±0.08
0.750	9.45 ±0.01	11.76 ±0.14	14.36 ±0.24	15.63 ±0.04
0.996	7.96 ±0.02	9.86 ±0.07	11.99 ±0.25	14.03 ±0.07

[a] Mean values for two runs except for the highest buffer measurement at $n = 0.000$, which is the mean for five runs. [b] Ionic strength maintained at 1.00 by added potassium chloride.

k_{obsd} to acetate concentration according to eq 1, are given in Table II.

$$k_{obsd} = k_W + k_B[NaO_2CCH_3] \quad (1)$$

Proton Inventories.[8] Figure 1 exhibits plots of both k_W and k_B from eq 1 as a function of the atom fraction of deuterium (n) in the solvent. The n dependence of k_W is a subject of this paper and is discussed in detail below. A discussion of $k_B(n)$ will be published with related work at a later time but it can be noted here that, as the arbitrarily drawn line in the figure emphasizes, the situation is complex. The faster rates observed for mixtures than for either pure isotope solvents are very probably real and mechanistically significant.

Table II.[a] Catalytic Rate Constants for Hydrolysis of 1.84×10^{-5} *M* *O*-Acetyl-3,5-dinitrosalicylic Acid in Binary Mixtures of Protium and Deuterium Oxides (Atom Fraction of Deuterium n) at $25.00 \pm 0.01°$ (μ 1.00)

n	$10^4 k_W$, sec^{-1}	$10^4 k_B$, M^{-1} sec^{-1}
0.000	1.296 ± 0.007	1.29 ± 0.01
0.250	1.037 ± 0.020	1.37 ± 0.04
0.375	1.018 ± 0.005	1.28 ± 0.01
0.500	0.852 ± 0.007	1.33 ± 0.01
0.625	0.818 ± 0.011	1.22 ± 0.02
0.750	0.752 ± 0.339	1.20 ± 0.08
0.996	0.588 ± 0.006	1.16 ± 0.01

[a] See eq 1 for definitions.

Discussion

The incorporation of 39% of the ^{18}O originally present in labeled water solvent into the carboxyl group of the salicylate product in the hydrolysis of *O*-acetyl-3,5-dinitrosalicylic acid, observed by Fersht and Kirby,[4] shows unambiguously that some attack of water (k_W reaction) is occurring at the salicylate carbonyl, presumably of the anhydride **2**. The combination of this evidence with the normal solvent isotope effect of 2.1 suggested[4] transition state structure **3**. However, to the degree that linearity is important[9] in the O···H···O bonding system shown in **3**, there is some stereochemical difficulty with it. Figure 2 illustrates the essential problem in the form of scale drawings of reactant-like and product-like transition state structures for intramolecularly catalyzed water attack at the salicyl carbonyl of **2**. In neither case can linearity of the catalytic hydrogen bond be achieved simultaneously with good orbital overlap in the forming OC bond. On the other hand, an excellent stereochemistry is available for water attack at the distal, acetyl carbonyl group; in fact, the structure is an isostere, or stereochemical equivalent, of structure **4**, for which a one-proton bridge has already been established.[5] It is possible that as much as 61% of the reaction proceeds by this route, since the labeling experiment cannot distinguish this path from that of transition state **3**. If indeed the entire reaction goes by way of the anhydride **2**, then the observed solvent isotope effect should be the weighted average of an effect arising from a one-proton catalytic

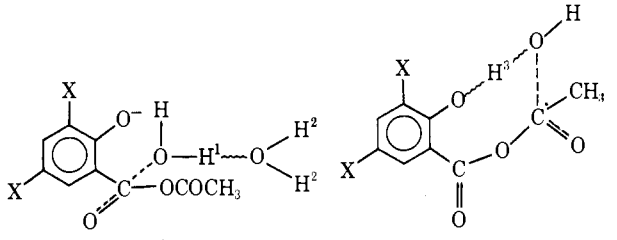
(9) Several lines of evidence indicate that *catalytic* hydrogen bridges strongly prefer a linear structure [R. D. Gandour, *Tetrahedron Lett.*, 295 (1974)] although exceptions may occur [M. I. Page and W. P. Jencks, *J. Amer. Chem. Soc.*, **94**, 8818, 8828 (1972), interpret their findings as indicating a low degree of rigidity in a transition state probably involving a hydrogen bridge]. Whether stable hydrogen bonds constitute good models for catalytic hydrogen bridges is unclear, but in any case the tendency of stable hydrogen bonds to attain linearity is controversial (*cf.* recent discussions by P. A. Kollman and L. C. Allen, *Chem. Rev.*, **72**, 283 (1972); G. C. Pimentel and A. L. McClellan, *Annu. Rev. Phys. Chem.*, 347 (1971); W. C. Hamilton and J. A. Ibers, "Hydrogen Bonding in Solids," W. A. Benjamin, New York, N. Y., 1968; and J. Donohue, W. C. Hamilton, and B. Kamb in "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Ed., W. H. Freeman, San Francisco, Calif., 1968). New studies by D. H. Aue, H. M. Webb, and M. T. Bowers, *J. Amer. Chem. Soc.*, **95**, 2699 (1973), and R. Yamdagni and P, Kebarle, *ibid.*, **95**, 3504 (1973), show that intramolecular hydrogen bonds in the gas phase are much stronger when linear.

bridge for attack at the acetyl carbonyl **5b** and an effect from *uncatalyzed* hydrolysis (or electrostatically catalyzed hydrolysis) of the anhydride linkage by attack at the salicyl carbonyl.[10] The study by Batts and Gold of acetic anhydride hydrolysis[6] suggests structure **5a** for proximal carbonyl attack. They found the normal solvent isotope effect of 2.88 to come from three protons, presumably two of a base-catalytic water molecule and one bridging proton. The former (H^2 in **5a**) denotes normal effects of 1.2 each and the bridge a normal effect of 2. If we simply adopt these values of Batts and Gold's work, assuming no difference in isotope effects between **1** and acetic anhydride, we can calculate from eq iv of ref 10 the requisite isotope effect for the one-proton bridge H^3 of structure **5b** in order to generate the observed weighted average of 2.2. We find a normal effect of 1.9, exactly in the range 2.2 ± 0.5 which we suggested to be typical of one-proton solvation bridges.[5] Thus, this model is readily capable of accounting for the gross solvent isotope effect and we now test it against the proton inventory.

Using the terminology of ref 10, we write eq 2 for the

$$k_W{}^n = k_p{}^n + k_d{}^n \qquad (2)$$

rate constant $k_W{}^n$ in a solvent of deuterium atom fraction n. From structures **5a** and **5b** and the general



5a **5b**

eq 3 for $k_n(n)$, when reactants are equally stable in

$$k_n = k_0 \prod_i^{\nu} (1 - n + n\phi_i) \qquad (3)$$

protium oxide and deuterium oxide[8] and there are ν exchangeable hydrogenic sites in the transition state, each with its characteristic isotopic fractionation factor[8] $\phi_i \equiv \{([D]_i/[H]_i)/(n[1 - n])\}$, we write eq 4. Note that in eq 4, the isotope effect on k_p is assumed to arise

$$k_W{}^n = k_p{}^0(1 - n + n\phi_1)(1 - n + n\phi_2)^2 + k_d{}^0(1 - n + n\phi_3) \qquad (4)$$

from one bridging proton (ϕ_1; H^1 in **5a**) and two protons of the catalytic water molecule (ϕ_2; H^2 in **5a**), while the isotope effect on k_d is taken as originating in a one-proton bridge (ϕ_3; H^3 in **5b**). From eq 4, we factor out $k_W{}^0$ to obtain eq 5a. If our simple model is

$$k_W{}^n = k_W{}^0[(k_p{}^0/k_W{}^0)(1 - n + \eta\phi_1)(1 - n + n\phi_2)^2 + (k_d{}^0/k_W{}^0)(1 - n + n\phi_3)] \qquad (5a)$$

(10) The argument for the weighted average is as follows. Let k^H and k^D be the total rate constants in protium and deuterium oxides, respectively, and $k^L{}_p$ and $k^L{}_d$ be the rate constants for attack at proximal (*i.e.*, salicyl) and distal (acetyl) carbonyl groups, respectively, in solvent L_2O. Then $k^L = k^L{}_p + k^L{}_d$. Also

$$k^D{}_p = [(k^H{}_p/k^H)(k^D{}_p/k^H{}_p)]k^H \qquad (i)$$

$$k^D{}_d = [(k^H{}_d/k^H)(k^D{}_d/k^H{}_d)]k^H \qquad (ii)$$

by identity. Combining (i) and (ii) to form k^D and taking[4] $k^H{}_p/k^H = 0.39$ and $k^H{}_d/k^H = 0.61$

$$k^D = 0.39(k^D{}_p/k^H{}_p)k^H + 0.61(k^D{}_d/k^H{}_d)k^H \qquad (iii)$$

$$(k^D/k^H) = 0.39(k^D{}_p/k^H{}_p) + 0.61(k^D{}_d/k^H{}_d) \qquad (iv)$$

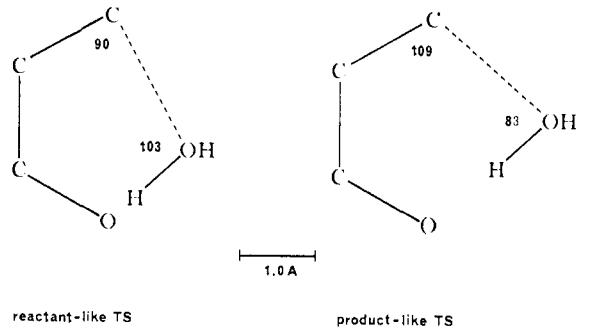


Figure 2. Limiting structures, drawn to scale, for reactant-like and product-like transition states for water attack at the proximal (salicyl) carbonyl group of **2**, with linear proton bridging to the phenoxy center. The full bonds are indicated by solid lines (aromatic CC, 1.40 Å; aromatic carbonyl CC, 1.45 Å; CO, 1.35 Å; OH, 0.96 Å; angles of 109 and 120° for bonds and orbitals). The carbonyl group is taken as trigonal in the reactant-like and tetrahedral in the product-like structure. In both cases, the COH angle on the phenoxy side of the hydrogen bond is fixed at 109°, which determines the length of the partial (dotted) CO bonds. These are (fortuitously) equal, at 2.0 Å, in both cases. Both structures result in extraordinarily short O–O distances (1.45 Å in the reactant-like structure, 2.2 Å for the product-like structure). The former is unacceptable [*cf.* C. N. R. Rao in "Water," F. Franks, Ed., Vol. 1, Plenum Press, New York, N. Y., 1972, pp 102–103], while in the latter case, even if catalytic interaction produced a very short O–O distance, reduction in overlap in the forming C–O from the unfavorable bond angle of 83° should enormously strain the structure.

$$k_W{}^n = k_W{}^0[0.39(1 - n + 0.50n)(1 - n + 0.83n)^2 + 0.61(1 - n + 0.52n)] \qquad (5b)$$

correct, then $k_p{}^0/k_W{}^0 = 0.39$, $k_d{}^0/k_W{}^0 = 0.61$, $\phi_1 = 0.50$, $\phi_2 = 0.83$ (both taken from Batts and Gold[6]), and $\phi_3 = 0.52$ (1/1.9). Substitution yields eq 5b, which is plotted as the solid line in Figure 1 with $10^4 k_W{}^0 = 1.27$ sec^{-1}. The fit is obviously adequate and we conclude that it is possible to explain the mild curvature of $k_W{}^n(n)$, visually apparent in Figure 1, by this means. Furthermore, it would seem that the overall solvent isotope effect of 2.2 arises from contributions of 39% from water-catalyzed anhydride hydrolysis at the proximal center ($0.39 \times 2.88 = 1.12$) and 61% from intramolecular-phenoxide-catalyzed attack of water at the *distal* carbonyl function ($0.61 \times 1.9 = 1.15$).[11]

This study, together with Minor's investigation[5] of the dichloroaspirin reaction, constitutes a useful illustration of the value of the proton-inventory technique. The numerical equality of the two solvent isotope effects originally gave rise to postulation of the two simplest transition states **3** and **4** by straightforward reasoning.[4] Only when the dinitro substrate gave a curved proton-inventory plot, while the aspirin-like substrate had

(11) Although the curvature in $k_W{}^n(n)$ is obviously rather small, a generalized quadratic least-squares fit to the data [polynomial regression using program BMDO5R (version of August 16, 1965; Health Sciences Computing Facility, UCLA; obtained from the Computation Center, University of Kansas)] is significantly better (rms deviation 0.36) than the best linear fit (rms deviation 0.40); the quadratic term is statistically significant at the 80% confidence level (Variance-Ratio or F-test: R. A. Fisher and P. Yates, "Statistical Tables for Biological, Agricultural and Medical Research," 5th ed, Oliver and Boyd, London, 1957). The fit of eq 5b is better than linear and not as good as the least-squares quadratic when the sum of squared deviations is considered (linear, 0.82; eq 5b, 0.61; least-squares quadratic, 0.51), but when degrees of freedom are included, and it is remembered that only $k_W{}^0$ is determined by fitting in eq 5b, then eq 5b gives the best fit of all (rms deviations: linear, 0.40; least-squares quadratic, 0.36; eq 5b, 0.32).

produced a linear plot, did it emerge that a structure other than **3** would be required. Stereochemical considerations[9] then led to the postulation of **5a** and **5b** and to the test of these against the proton inventory as shown in Figure 1. Postulations of catalytic interactions stereochemically equivalent to that in **3** have not been uncommon although transition state structures have not been established for any such case, simplicity apparently having been generally the guiding principle. It is not yet clear whether the nonlinear catalytic bridges which would be required in such structures are truly "forbidden" or are sufficiently accessible in energy that other factors could compensate for their inclusion. The proton-inventory technique may on occasion serve to discriminate among these possibilities. It should be noted that both pathways (*via* **5a** and **5b**) are probably catalytic routes, **5b** having a solvation bridge[5] and **5a** enjoying intramolecular electrostatic stabilization.

Experimental Section

Materials. Sodium acetate and acetic acid were purified[12] and stored in a desiccator until needed. Potassium chloride, Analytical Grade, was dried in an oven and stored in a desiccator until needed. Acetyl-3,5-dinitrosalicylic acid was prepared according to the previous procedure.[4] Purification was accomplished by low-temperature recrystallization from ether and gave material with mp 92.5–94.0° (lit.[4] 93–94°).

Anal. Calcd for $C_9H_6N_2O$: C, 40.00; H, 2.22; N, 10.38. Found: C, 39.73; H, 2.21; N, 10.27.

Buffer Solutions. Solvents were prepared from distilled water which had been passed through a mixed-bed ion-exchange column and 99.8% deuterium oxide (Diaprep Corp.). Mixed isotopic solvents were prepared gravimetrically. Addition of buffer components, potassium chloride, and acetyl-3,5-dinitrosalicylic acid had a negligible effect (<0.2%) on the mole fraction of deuterium in the mixed solvents. The atom fraction of deuterium in the "pure" deuterium oxide was determined by an nmr technique in which the integral of the water protium signal was compared to the integral of a protium signal from dioxane in known concentration. The solutions were prepared volumetrically and all components of every buffer solution determined gravimetrically. The pH(D) was measured with a pH meter as a check.

Kinetics. The release of 3,5-dinitrosalicylate ion at 337 nm was followed with a Cary 16 ultraviolet-visible spectrophotometer equipped with a constant temperature apparatus. The reaction was initiated by injecting 100 μl of a stock solution of dinitroaspirin in the appropriate solvent mixture into 3.00 ml of the thermally equilibrated buffer solution in a 3-ml quartz cuvette. The cuvettes were kept in the thermostated bath and briefly (<50 sec) removed for null determination of the absorbance in the thermostated cell compartment. At least 20 points were determined for each run and the rate for each buffer concentration was measured in duplicate. The reaction was followed for at least three half-lives and the first-order rate constants were calculated by a nonlinear least-squares computer program from given time and absorbance values. Internal standard deviations of the rate constants (within one run) were consistently less than ±0.8%.

(12) R. K. Birdwhistell and E. Griswold, *J. Amer. Chem. Soc.*, 77, 873 (1955).

Rigid Active Esters in Peptide Synthesis[1]

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Abstract: The rates of aminolysis of *o*-nitrophenyl esters of protected amino acids are higher, less solvent dependent, and less sensitive to steric hindrance than the aminolysis rates of the corresponding *p*-nitrophenyl esters. The higher reactivity of the ortho isomers can be readily explained by the electron-withdrawing effect of the nitro group, which is more efficient in the ortho than in the para position. The differences observed in the influence of solvents and of steric hindrance were traced to an intramolecular interaction between the nitro group and the amide group in *o*-nitrophenyl esters. The resulting rigid, cyclic conformation is revealed in the high optical activity and a pronounced effect of temperature on the rotation of a series of *o*-nitrophenyl esters.

At the time of the introduction of nitrophenyl esters[3] as reactive intermediates in peptide synthesis, the esters of *o*-, *m*-, and *p*-nitrophenol and also of 2,4-dinitrophenol were examined. The choice of *p*-nitrophenyl esters as practical reagents was dictated by their relative readiness to crystallize. The more reactive 2,4-dinitrophenyl esters were not favored because of their sensitivity to hydrolysis. Many years later, when the application of active esters in solid-phase peptide synthesis was studied,[4,5] *p*-nitrophenyl esters were found, contrary to earlier, preliminary observations of Merrifield,[6] useful in the preparation of peptides on a solid support. Nevertheless, only the *p*-nitrophenyl esters of (protected) asparagine and glutamine were generally accepted for this purpose, because by acylation with the purified active esters dehydration of the side-chain carboxamides[7] can be avoided. The reluctance toward adopting active esters of other amino acids is probably due to the moderate reaction rates obtainable with *p*-nitrophenyl esters: rates that lag considerably behind those of coupling with dicyclohexylcarbodiimide.[8] A seemingly easy solution to this difficulty would be the use of more reactive esters such as penta-

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(2) Overseas Research Scholar sponsored by the Ministry of Education in Japan.

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