in a manner analogous to those shown earlier for ethylene, that is, butene complexation and insertion into the Ni–R bond followed by β-hydride elimination. Similar proposals have been expressed earlier for the dimerization of ethylene by (η^1-aryl)NiBr(PPh_3)$_3$–BF_3OEt_2 where a Ni–H species was formed from the dissociation of ethylene (coordinated ethylene did not insert into the η^1-aryl–Ni bond).[18]

Our experiments have also shown that the reaction rate is very sensitive to solvent and that electron-demanding arene solvents cause the fastest reaction. This requires that the solvent be involved in the coordination sphere but that the solvent molecule be highly labile. This is indeed the case for η^6-halobenzenes on Ni(II) and Co(II).[4]

The cis/trans ratio is dependent on solvent and on the 1-C_4H_8/Ni ratio. This would imply a steric effect of the solvent and a sensitivity to whether more than one butene molecule is coordinated. Actually, high concentrations of 1-C_4H_8 not only affect the cis/trans ratio but also cause decomposition of the Ni complex. Apparently the coordination of a second butene molecule changes the reaction pathway significantly.

The original η^6-arene affects both the rate and the cis/trans ratio (in the same solvent). This observation suggests that the original arene is only partially displaced by solvent arene. Indeed, our earlier work has shown that very large excesses of halobenzenes are necessary to displace even a small amount of η^6-benzene or η^6-toluene.[4b] Thus, if the toluene complex were placed in a bromobenzene solvent, a small amount of toluene, perhaps less than 1%, would be displaced at equilibrium.

The cis/trans ratio under similar conditions does not change when comparing SiF_3 and $SiCl_3$ derivatives. However, the C_6F_5 derivative does yield a higher cis/trans ratio when all are compared in toluene solution. However, in bromobenzene solution the differences disappear, indicating that the active catalyst in bromobenzene is not as sensitive to steric factors as the active catalyst in toluene.

(18) Maruya, K.; Mizoroki, T.; Ozaki, A. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 2255.

The activity order of (η^6-arene)NiR_2 (R = $SiCl_3$ > SiF_3 > C_6F_5) reflects in part the strength of the η^6-arene–M bond (where R = $SiCl_3$ the η^6-arene–Ni bond is longest).[19] The fact that (η^6-arene)$Co(C_6F_5)_2$ is not active while (η^6-arene)$Ni(C_6F_5)_2$ is somewhat active also reflects the strength of the η^6-arene bond, the Co system having a shorter stronger η^6-arene–M bond than the Ni system.[20]

The following mechanistic sequence (Scheme II) attempts to account for all of the observations, which summarized are the following:[21] (1) more strongly bound original arene affects the rate, (2) ethylene and water experiments suggest a Ni–H intermediate, (3) arene solvents strongly affect the rate (electron demanding and thereby weakly π-bonding arene solvents give higher rates), (4) only one butene molecule is involved in the coordination sphere (in the absence of high concentrations of 1-butene), (5) 1-butene is involved in both an initiation step (therefore an induction period) and a termination step (decomposition of the catalytic species, especially at higher 1-butene concentrations), and (6) steric effects cause changes in the cis/trans product ratio.

Acknowledgment. The support of the National Science Foundation is gratefully acknowledged.

Registry No. (η^6-Toluene)$Ni(SiCl_3)_2$, 80410-01-1; (η^6-benzene)$Ni(SiCl_3)_2$, 89389-59-3; (η^6-mesitylene)$Ni(SiCl_3)_2$, 100909-73-7; (η^6-toluene)$Ni(SiF_3)_2$, 88083-21-0; (η^6-toluene)$Ni(C_6F_5)_2$, 66197-14-6; 1-butene, 106-98-9; bromobenzene, 108-86-1; 3-bromotoluene, 591-17-3; 4-bromotoluene, 106-38-7; 2-chlorotoluene, 95-49-8; chlorobenzene, 108-90-7; 3-chlorotoluene, 108-41-8; toluene, 108-88-3; tetrahydrofuran, 109-99-9; *n*-heptane, 142-82-5; iodobenzene, 591-50-4; anisole, 100-66-3; fluorobenzene, 462-06-6; methylene chloride, 75-09-2.

(19) Private communication with Professor Lewis Radonovich, who has completed the X-ray structural determinations.

(20) Radonovich, L. J.; Klabunde, K. J.; Behrens, C. B.; McCollor, D. P.; Anderson, B. B. *Inorg. Chem.* **1980**, *19*, 1221.

(21) Attempts to characterize intermediate Ni–H species by ^1H and ^{13}C NMR were not successful. This is probably due to the short lifetime of such species, or due to intermediate paramagnetic species present that would severely broaden NMR resonances (paramagnetic materials are present according to ESR studies of precipitated decomposition products).

Intramolecular General Acid and General Base Catalyses in the Hydrolysis of 2-Halotryptophans and Their Analogues

Robert S. Phillips and Louis A. Cohen*

Contribution from the Laboratory of Chemistry, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205.
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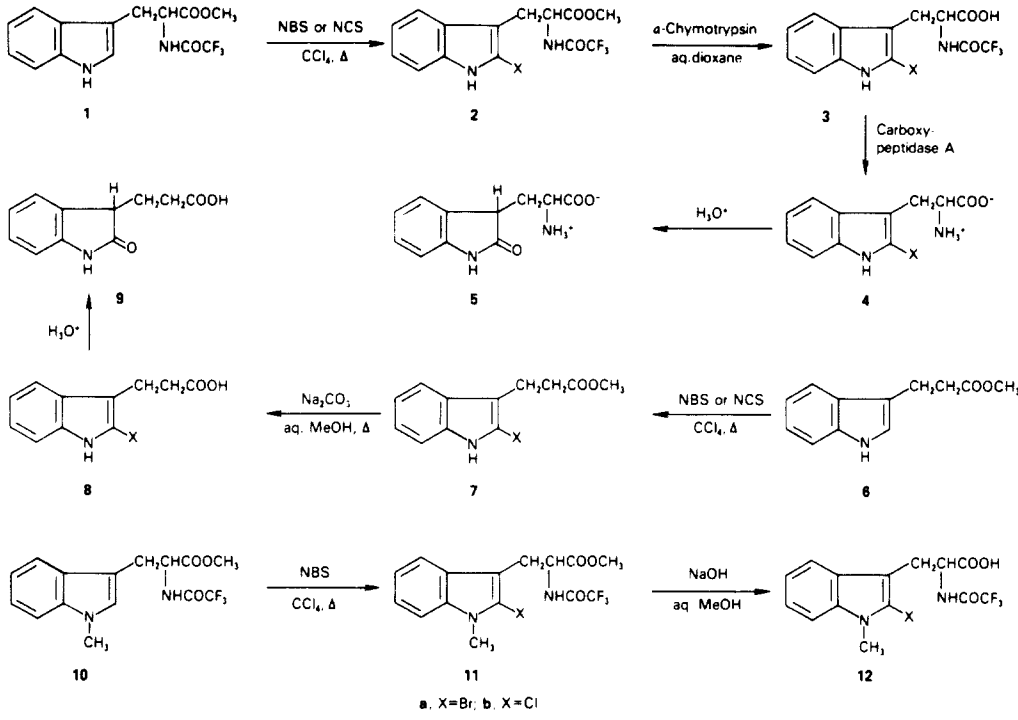
Abstract: Radical halogenation of protected L-tryptophan, and of related indoles, leads to the corresponding 2-halo derivatives in high yield; enzymatic removal of the blocking groups provides the new amino acid analogues. In the concentration range 0.01–3.0 M $HClO_4$, hydrolysis to oxindoles of the fully protected 2-halotryptophans, as well as of 2-halo-3-(*R*)-indoles in general (R = CH_3, CH_2CO_2Et, $CH_2CH_2CO_2Me$), increases linearly with acidity (h_0) and gives extrapolated intercepts ≅0. Protonation of the indole at C-3 is relatively fast and addition of water to the resulting 2-haloindoleninium ion is rate-limiting. For α-*N*-acyl-2-halotryptophans and 2-haloindole-3-propionic *acids*, the extrapolated intercepts are >0 and additional pathways for hydrolysis must be invoked. For the latter compounds, k_{obsd} is much greater than for 2-haloskatoles in weakly acidic media, the factor increasing with pH to $>10^5$ at pH 5. In the pH region −0.5 to 1.5, enhanced hydrolysis is due, primarily, to intramolecular general base catalysis of water addition by carboxylate ion; above pH 1.5, such catalysis becomes so effective that indole protonation is now rate-limiting. Above pH 4, intramolecular proton transfer from the carboxyl group (general acid catalysis) is more effective than transfer from external hydronium ion by a factor of 127 M. On the other hand, the effective molarity for intramolecular general base catalysis of water addition is estimated to be $>10^{12}$. In the case of 2-haloindole-3-acetic acids, the transition-state geometry for general base catalysis is even more favorable and water addition to the protonated indolenine is so fast that indole protonation remains rate-limiting even in 1 M acid.

The widespread occurrence of indoles in both plant and animal metabolites has provided the primary stimulus in the search for novel or more effective drugs based on this ring system. A lack of synthetic routes has limited the number of 2-substituted bioindoles available for study;[1,2] halo derivatives have been particularly inaccessible, both because of their labilities in acid media[3]

(1) Phillips, R. S.; Cohen, L. A. *Tetrahedron Lett.* **1983**, *24*, 5555–5558.

Scheme I



and because halogen often appears to attack C-3 preferentially.[3] We have recently reported the preparation of the novel 2-bromo- and 2-chloro-L-tryptophans (**4**, Scheme I), and have provided preliminary data on the acid-catalyzed hydrolysis of these compounds.[1] As an adjunct to investigations on the biological properties of tryptophan analogues, we had undertaken a detailed analysis of the hydrolysis mechanisms of these and related 2-haloindoles. Our results indicate that hydrolysis occurs via the 2-haloindolenine tautomer but that the rate-limiting step changes with pH; of added interest, moreover, are the demonstrations of facile intramolecular proton transfer from a side-chain carboxyl group to C-3 and catalysis of water addition by the resulting carboxylate ion. The application of the first phenomenon in the design of transition-state inhibitors of tryptophan-metabolizing enzymes has already been developed.[4]

Results and Discussion

Preparation of 2-Haloindoles. In contrast to the extensive literature on the reactions of indoles with halogenating agents under polar, electrophilic conditions,[3,5] few reports have appeared on reactions under conditions that may favor radical substitution. Esters of indole-3-acetic acid[6] and skatole[7] have been halogenated with *N*-halosuccinimides in CCl_4. We have found that a number of other substituted indoles, including side-chain protected tryptophan, can be halogenated in good to excellent yield under the same conditions.[1]

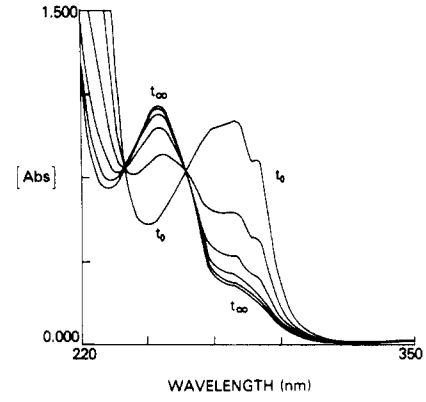


Figure 1. Changes in UV spectra (at 30-min intervals) for the hydrolysis of 2-bromoindole-3-propionic acid (**8a**) at pH 1.

Solubility and purification considerations dictate the use of maximally protected starting materials; e.g., **8** was obtained by halogenation of **6** and saponification of the resulting halo ester **7**. Since the acid sensitivity of the 2-haloindoles has been well documented,[3] the use of the trifluoroacetyl group to protect the α-nitrogen of tryptophan was originally dictated by the need for a base-labile protecting group; subsequent results, however, showed this group to be critical for the success of the reaction since α-*N*-acetyl-L-tryptophan methyl ester gave no significant yield of halogenated product under our conditions. In contrast to the results with **7**, alkaline hydrolysis of **2** did not proceed cleanly to the free amino acid; instead, the protecting groups had to be removed sequentially by treatment with α-chymotrypsin and carboxypeptidase A. Thus, it would appear that the formation of a number of side products (some colored) during the hydrolysis of **2** involves the participation of the liberated side-chain amino group. Surprisingly, **11** could be converted readily to the corresponding amino acid under alkaline conditions—without significant byproduct formation; thus, the base sensitivity of **4** may also depend on ionization of N-1 in strongly alkaline media.

The fact that each of these halogenated indoles is hydrolyzed to an oxindole[5,9] is not sufficient proof that our products are

(2) Sundberg, R. J. "The Chemistry of Indoles"; Academic Press: New York, 1970.
(3) (a) Lawson, W. B.; Patchornik, A.; Witkop, B. *J. Am. Chem. Soc.* **1960**, *82*, 5918–5923. (b) Hinman, R. L.; Bauman, C. P. *J. Org. Chem.* **1964**, *29*, 1206–1215. (c) Powers, J. C. *Ibid.* **1966**, *31*, 2627–2631. (d) For a review of halogenated indoles, see: Powers, J. C. In "The Chemistry of Heterocyclic Compounds. Indoles, Part Two"; Houlihan, W. J., Ed.; Wiley: New York, 1972; pp 127–178.
(4) Phillips, R. S.; Miles, E. W.; Cohen, L. A. *Biochemistry* **1984**, *23*, 6228–6234.
(5) (a) Patchornik, A.; Lawson, W. B.; Gross, E.; Witkop, B. *J. Am. Chem. Soc.* **1960**, *82*, 5923–5927. (b) Ohno, M.; Spande, T. F.; Witkop, B. *Ibid.* **1968**, *90*, 6521–6522; (c) *Ibid.* **1970**, *92*, 343–348. (d) Kobayashi, T.; Inokuchi, N. *Tetrahedron* **1964**, *20*, 2055–2058. (e) Hino, T.; Nakagawa, M.; Wakatsuki, T.; Ogawa, K.; Yamada, S. *Ibid.* **1967**, *23*, 1441–1450. (f) Troxler, F.; Hofmann, A. *Helv. Chim. Acta* **1957**, *40*, 2160–2170.
(6) Porter, W. L.; Thimann, K. V. *Phytochemistry* **1965**, *4*, 229–243.
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(8) Jackson, A. H.; Smith, P. *Tetrahedron* **1968**, *24*, 2227–2239.
(9) Hinman, R. L.; Lang, J. *J. Am. Chem. Soc.* **1964**, *86*, 3796–3806.

Table I. Kinetic Parameters for Hydrolysis of 2-Haloindoles under Strongly Acidic Conditions

compd	10^5k_H,[a] M^{-1} s^{-1}	10^4k_{int},[b] s^{-1}	compd	10^5k_H,[a] M^{-1} s^{-1}	10^4k_{int},[b] s^{-1}
2-bromoskatole	1.0	0[c]	**12a**	10	6.6
ethyl 2-bromoindole-3-acetate	3.6	0	**4a**	4.4	2.8
7a	6.4	0	2-chloroskatole	1.4	0
2a	0.8	0	**2b**	1.4	0
2-bromoindole-3-acetic acid	1400[d]	0	**3b**	6.7	4.5
8a	20	4.4	**4b**	7.2	3.6
3a	3.2	2.4	**8b**	41	12

[a] Equivalent to k_4K_2 in eq 2. [b] Equivalent to k_3K_1 in eq 2. [c] Extrapolated y intercept is zero within experimental error in eq 2. [d] Equivalent to k_2.

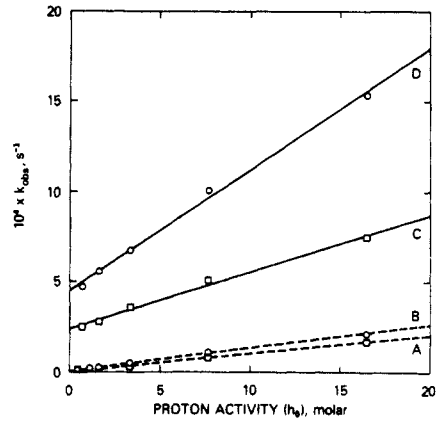


Figure 2. Dependence of rate of hydrolysis of 2-haloindoles on proton activity: (A) 2-bromoskatole; (B) 2-chloroskatole; (C) α-*N*-(trifluoroacetyl)-2-bromo-L-tryptophan (**3a**); (D) α-*N*-(trifluoroacetyl)-2-chloro-L-tryptophan (**3b**).

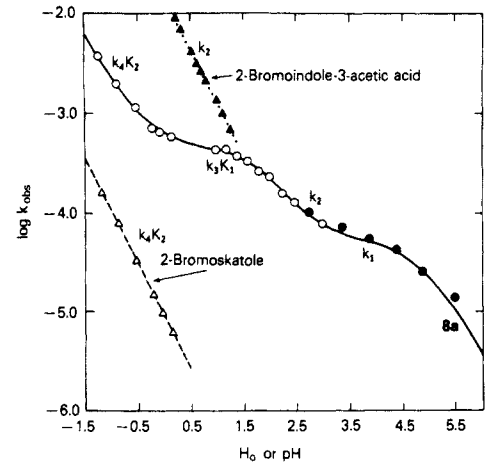


Figure 3. Dependence of log k_{obsd} for the hydrolysis of 2-haloindoles on H_0 or pH: 2-bromoindole-3-propionic acid (**8a**) in $HClO_4$ media (○), in acetate buffer media (●); solid line, curve calculated by use of eq 1 and kinetic parameters of Table IV; 2-bromoskatole (△); 2-bromoindole-3-acetic acid (▲).

2-haloindoles rather than 3-haloindolenines, since the halogen can undergo an acid-catalyzed migration to C-2;[2c,3d] however, NMR spectra clearly show the absence of an aldimine proton at low field[8] and the presence of a broad NH signal. Furthermore, UV spectra (Figure 1) are consistent with those of indoles and not of indolenines.[8]

Acid Hydrolysis of 2-Haloindoles. In agreement with previous reports,[3] we find that 2-haloindoles are subject to facile hydrolysis in acidic media, the products being the corresponding oxindoles.[5,9] The progress of these reactions is conveniently monitored by the decrease in absorbance at 280 nm as the indole chromophore is lost (Figure 1). Pseudo-first-order kinetics are obtained under the conditions of our measurements, with semilog plots linear through 4–5 half-lives. In addition, the repetitive spectra exhibit clean isosbestic points at about 235 and 260 nm, indicating that there is no significant buildup of intermediates (Figure 1). For 2-bromo- and 2-chloroskatole, the observed pseudo-first-order rate constants exhibit a linear dependence on proton activity (h_0) in the concentration range 0.01–3.0 M $HClO_4$ (Figure 2, slopes A and B), with extrapolated intercepts that are zero within experimental error. At concentrations of $HClO_4$ above 3.0 M, significant deviation from linearity of k_{obsd} was found (data not shown); even in the less acidic media, plots of k_{obsd} vs. $[H_3O^+]$ or the H_I scale developed for indoles[9] were markedly nonlinear.

Polyfunctional 2-haloindoles, such as **2** and **7**, are hydrolyzed to the oxindoles at rates comparable to those of the corresponding skatoles (Table I); however, **3** and **4** were found to be much more reactive. For the latter compounds, plots of k_{obsd} vs. proton activity exhibit large intercept values in addition to the expected linear dependence on h_0 (Figure 2, slopes C and D). Thus, the rate law for hydrolysis of **3** and **4** must include a term that is independent of catalysis by external acid. Similar large intercepts are obtained with 2-haloindole-3-propionic acids **8**, but not with their esters **7** nor with 2-bromoindole-3-acetic acid. We chose to use series **8** to investigate further the origin and mechanism of the apparent acid-independent reaction.

pH Dependence in the Hydrolysis of 8. The pH–rate profiles for acid hydrolysis of **8a,b** above pH 0 are complex (Figure 3). Two regions of inflection are evident for both compounds at about pH 1.5 and 4.8. While the latter value is consistent with ionization of the side-chain carboxyl group,[10] there is no ionizable function in these compounds with an expected pK value in the vicinity of 1.5.[9] A plot very similar to that of **8a** in Figure 3 was also obtained for the hydrolysis of the 1-methyl derivative **12a**. We then considered the possibility that the inflection at low pH might result from a change in rate-determining step. Solvent deuterium isotope effects are negligible at pH 1 and below (Table II), but the effect increases with pH and reaches a k_{H_2O}/k_{D_2O} value of 2.4 at pH 3. These results suggest that, while proton transfer may not be rate-limiting at pH 1, it is at least partly limiting at pH 3. This conclusion is supported by the results of the nucleophilic trapping experiments presented in Table III. Semicarbazide was selected to compete with water for halogen displacement at C-2 because of its weak basicity (pK 3.6) and because it was expected to be

Table II. Solvent Deuterium Isotope Effect on Rate of Hydrolysis of 2-Bromoindole-3-propionic Acid

pH	pD	10^4k_{obsd}, s^{-1}	k_{H_2O}/k_{D_2O}
1.0		4.7	
	1.0	4.0	1.2
2.0		2.6	
	2.0	1.3	2.0
3.0		1.1	
	3.0	0.45	2.4

Table III. Effect of Semicarbazide on Rate of Hydrolysis of 2-Bromoindole-3-propionic Acid

pH	semicarbazide 0.2 M	10^5k_{obsd}, s^{-1}	oxindole, %
2.68	−	11	100
2.70	+	10	56
3.26	−	6.9	100
3.13	+	7.4	33
3.61	−	6.1	100
3.70	+	6.2	17

(10) The pK_a for the parent compound, indole-3-propionic acid, was determined to be 4.7 ± 0.1 by spectrofluorometric titration.

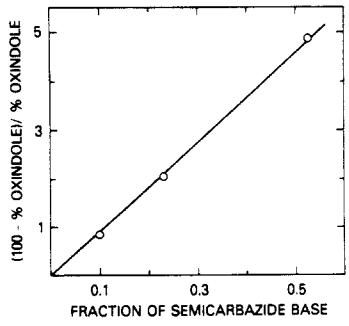
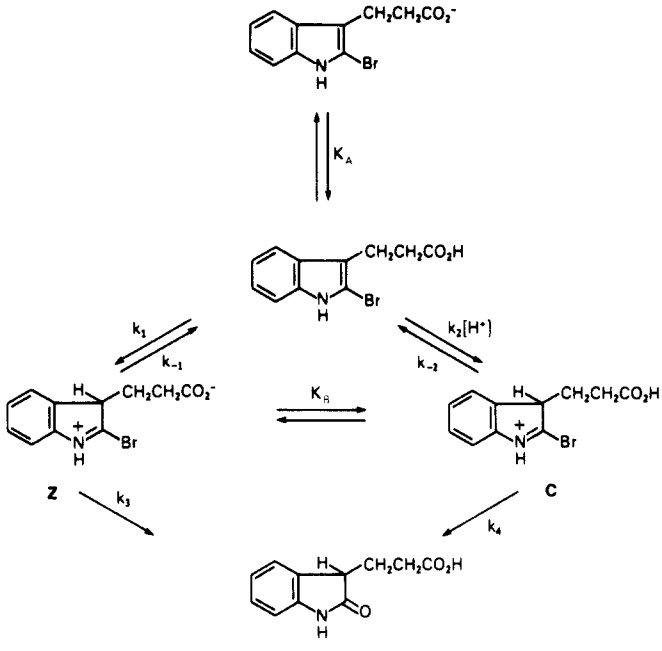


Figure 4. Effect of pH and variation in the fraction of neutral semicarbazide on the ratio of displacement of bromine in **8a** by semicarbazide and by water.

Scheme II



an effective α-nucleophile.[11] As the fraction of neutral semicarbazide increases with pH, the nitrogen nucleophile becomes more competitive and the fraction of oxindole-3-propionic acid (**9**) produced from **8a** decreases correspondingly; yet, the rate constant at each pH value was essentially unaffected by the addition of semicarbazide. Thus, the reaction with the nitrogen nucleophile must occur after the rate-determining step—at least within the pH range investigated. Furthermore, the linearity of a plot of the semicarbazone/oxindole ratio vs. fraction of neutral semicarbazide (Figure 4) confirms the uniformity of reaction mechanism within this pH range.

Mechanism of Hydrolysis of 2-Haloindoles. A minimal mechanism which is consistent with our results is presented in Scheme II. Acid-catalyzed hydrolysis of 2-haloindoles has been proposed to occur via initial formation of the 2-haloindolenines (intermediates C and Z in Scheme II).[3d] In the case of the 2-haloindole-3-propionic acids, the indolenine may arise via intramolecular proton transfer and lead to a zwitterionic intermediate (Z) or by proton transfer from solvent and lead to a cationic intermediate (C). Addition of water to the protonated indolenines (which may be facilitated by the carboxylate ion acting as a general base in the case of Z) would result in tetrahedral α-halocarbinolamine intermediates, from which the expulsion of halide ion should be fast. The duplication of the double inflection and general shape of Figure 3 for the hydrolysis of **12** appears to rule out any significant kinetic involvement of the neutral indolenine, even in the higher pH range. Nucleophilic participation by the side-chain carboxylate ion might also be considered. We have found no evidence, however, for the formaton of a transient enolic δ-lactone (of significant lifetime) by several criteria: (1) clear isosbestic points in the hydrolysis of **8a** (Figure 1);[12] (2) absence of unidentified materials in incomplete hydrolyses, according to TLC and HPLC; (3) absence of any mass spectral fragments attributable to the acylation of semicarbazide by an enolic lactone; (4) clear isosbestic points in the trapping experiments with semicarbazide.

Table IV. Kinetic Constants for 2-Haloindole-3-propionic Acids

substituent	k_1, s^{-1}	k_2, M^{-1} s^{-1}	k_3k_1, s^{-1}	k_4K_2, M^{-1} s^{-1}	pK_A
2-bromo (**8a**)	6.5×10^{-5}	3.3×10^{-2}	5.2×10^{-4}	2.0×10^{-4}	4.8
	$(3.9)^a$	$(1.9)^a$	$(1.0)^a$	$(1.2)^a$	
2-chloro (**8b**)	1.4×10^{-4}	4.3×10^{-2}	1.6×10^{-3}	4.1×10^{-4}	4.7

[a] Numbers in parentheses are the calculated isotope effects, k_{H_2O}/k_{D_2O}, for the particular kinetic parameter.

The rate law for the mechanism of Scheme II, with application of the steady-state approximation to Z, is shown in eq 1, where

$$k_{obsd} = (k_3K_1 + k_4K_2[H^+]) \times \left(\frac{k_1 + k_2[H^+]}{k_1 + k_3K_1 + k_2[H^+] + k_4K_2[H^+]}\right)\left(\frac{[H^+]}{[H^+] + K_A}\right) \quad (1)$$

$K_1 = k_1/k_{-1}$ and $K_2 = k_2/k_{-2}$. Under strongly acidic conditions, where we assume $k_2[H^+] >> k_1$, k_3K_1, and $k_4K_2[H^+]$, eq 1 simplifies to eq 2. Thus, in strong acid media, k_{obsd} exhibits a linear

$$k_{obsd} = k_3K_1 + k_4K_2[H^+] \quad (2)$$

dependence on proton activity,[13] with a slope equal to k_4K_2 and an intercept equal to k_3K_1 (Figure 2). At pH values above 0, $k_4K_2[H^+]$ will be negligible in value and the rate is described by eq 3. The data in Figure 3 were fitted to eq 1 by the use of the

$$k_{obsd} = k_3K_1\left(\frac{k_1 + k_2[H^+]}{k_1 + k_3K_1 + k_2[H^+]}\right)\left(\frac{[H^+]}{[H^+] + K_A}\right) \quad (3)$$

MLAB program on the NIH DEC-10 computer system.[14] A very good fit was obtained ($r > 0.99$) and the values of the parameters are given in Table IV. The values of k_3K_1 obtained by curve fitting (Table IV) are in quite reasonable agreement with those obtained by extrapolation to $h_0 = 0$ (Figure 2 and Table I). The values of K_A were determined separately by fit to the kinetic data above pH 3; the results are consistent with expectation for the γ-substituted propionic acids.[10]

Similar calculations were carried out by using data for kinetics in D_2O (in the pD range −0.5 to 3), providing the isotope effects on the intrinsic kinetic parameters (Table IV). We find a large isotope effect (3.9) on k_1, the rate constant for intramolecular proton transfer, and a relatively modest effect (1.9) on k_2, the rate constant for proton transfer from hydronium ion. Our results are similar to those obtained for the hydrolysis of enamines,[15] for which the isotope effect on general acid catalysis by acetic acid (9) is much greater than that for H_3O^+ (2.5). Furthermore, hydrolysis of vinyl ethers, which is subject to rate-limiting proton transfer, also shows larger isotope effects with carboxylic acids than with H_3O^+.[16]

Thus, our data indicate that, in the pH region above 3, the reactivity of 2-bromo(or 2-chloro)-indole-3-propionic acid is due to rate-limiting intramolecular proton transfer.[17] One measure of the efficiency of intramolecular catalysis is the effective molarity, EM (i.e., the ratio of the first-order rate constant for the

(11) (a) Sander, E. G.; Jencks, W. P. *J. Am. Chem. Soc.* **1968**, *90*, 6154–6162. (b) Dixon, J. E.; Bruice, T. C. *Ibid.* **1971**, *93*, 3248–3254, 6592–6597.

(12) For the δ-lactone, λ_{max} should occur at 260–270 nm; see: Nakagawa, M.; Hino, T. *Tetrahedron* **1970**, *26*, 4491–4503.

(13) For media below pH 1, a_{H^+} is substituted for $[H^+]$ in all equations.

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(15) Stamhuis, E. J.; Maas, W. *J. Org. Chem.* **1965**, *30*, 2156–2160.

(16) Kresge, A. J.; Chiang, Y. *J. Chem. Soc. B* **1967**, 58–61.

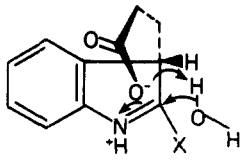
(17) The kinetic involvement of the protonated form of a very weak base, even at mild pH, has ample precedent; for example, see: Koehler, K.; Sandstrom, W.; Cordes, E. H. *J. Am. Chem. Soc.* **1964**, *86*, 2413–2419.

intramolecular reaction to that of the second-order rate constant for a corresponding intermolecular reaction).[18] The simplest comparison is based on the ratio of k_{obsd} for hydrolysis of **8a** (Figure 3) to that for hydrolysis of 2-bromoskatole at the same pH value (extrapolated from k_H in Table I): EM will then increase from 17 M at pH −1.5 to 1.2×10^6 M at pH 5.5. Considering, however, that a more valid comparison should involve an external acid of pK comparable to that of **8a**, we measured the very slow hydrolysis of 2-bromoskatole in 1 M aqueous acetic acid at pH 2.5 ($k_{obsd} \cong 6 \times 10^{-8}$ M^{-1} s^{-1}). Comparison with k_{obsd} for hydrolysis of **8a** at pH 2.5 (1.2×10^{-4} s^{-1} provides an EM of 2×10^3 M. Since the rate-determing step for the hydrolysis of 2-bromoskatole is water addition to the indolenine rather than proton transfer, we actually have no inter- and intramolecular reactions with parallel mechanisms and, thus, cannot obtain a true EM. For the case of **8a** itself, intermolecular proton transfer by H_3O^+ (k_2 in Table IV) is 3.3×10^{-2} M^{-1} s^{-1}; intramolecular proton transfer (k_1) is 6.5×10^{-5} s^{-1}. By including a correction for the difference in pK_a of the carboxylic acid (4.8) and hydronium ion (−1.74), and taking a Brønsted α value of 0.74,[19] we arrive at EM = 6.5×10^{-5} $s^{-1}/((3.3 \times 10^{-2}$ M^{-1} $s^{-1}) \times 10^{-6.5\alpha}) = 127$ M. This value is somewhat larger than those obtained for most other systems involving intramolecular general acid catalysis,[18] EM's being generally 55 M or less. For example, in the hydrolysis of a vinyl ether substituted norbornanecarboxylic acid, the effective molarity was only 1.3 M.[20] On the other hand, the hydrolyses of acetals derived from salicylic acid have been reported to exhibit EM's of 4.6×10^3 to 2.9×10^4 M.[18,20b,c]

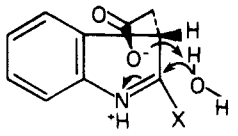
Under strongly acidic conditions, the hydrolysis of 2-haloindoles evidently occurs via rate-limiting attack of water on the 2-haloindolenine cation. Thus, for hydrolysis in HCl or DCl solution, there is no significant isotope effect either for 2-bromoskatole (data not shown) or for **8a** (Table IV). Furthermore, the UV spectrum of 2-bromoskatole in 60% $HClO_4$ (taken within a few seconds of mixing with the use of an HP8450 diode array spectrophotometer) is very similar to that of skatole itself under these conditions, indicating rapid formation of the indolenine cation.[9] The second-order rate constant for the hydrolysis of 2-chloroskatole was found to be invariant throughout the H_0/pH range investigated (−1.5 to 2.5), suggesting that a change in rate-determining step does not occur for skatoles in the same region (pH 1–2) in which it occurs for the indole-3-propionates.

As mentioned previously, the attack of water on the zwitterionic indolenine (Z in Scheme II) appears to be facilitated by the side-chain carboxylate ion acting as a general base. The rate constant for external protonation of **8a** (3.3×10^{-2} M^{-1} s^{-1}, k_2 in Table IV) and that for 2-bromoindole-3-acetic acid (1.4×10^{-2} M^{-1} s^{-1}, k_H in Table I) are very similar. Since the basicity of C-3 in 2-bromoskatole should not differ significantly from the values for these analogues, there is no reason to believe that the rate constant for protonation of 2-bromoskatole should be significantly different either. Thus, the change in rate-determining step for **8a** above pH 1 must result from a great increase in the rate constant for water addition to the protonated indolenine and such enhancement can be attributed only to intramolecular catalysis by carboxylate ion. Indeed, Dreiding or space-filling models suggest that the conformation suitable for intramolecular proton transfer from carboxyl to C-3 in **8** is *equally* suitable for catalysis of water addition by the resulting conjugate anion (A). The very same functional group may be operating consecutively as general acid catalyst and as general base catalyst without significant relocation, making **4** and **8** very interesting models for enzyme catalysis.

In the case of general base catalysis, effective molarity can be estimated from rate data for reactions with parallel mechanisms.



A



B

The hydrolysis of 2-bromoskatole in 1 M aqueous acetic acid shows $k_{obsd} = 3 \times 10^{-8}$ M^{-1} s^{-1}, *after* correction for the hydronium ion catalyzed rate at pH 2.5. Since the rate-determining step for hydrolysis of the 2-haloskatoles is water attack on the protonated indolenine, the rate acceleration observed in the presence of acetic acid must be due to general base catalysis by acetate ion—rather than general acid catalysis by acetic acid. After adjustment for the concentration of available acetate ion at pH 2.5, $k_{AcO^-} = 4.7 \times 10^{-6}$ M^{-1} s^{-1}. From the kinetic analysis of the pH profile for **8a**, we obtain the composite rate constant, k_3K_1, for water addition to the zwitterionic intermediate Z. From the thermodynamic cycle $K_1 = K_BK_2$, $K_1 \cong (1.6 \times 10^{-5})(7.9 \times 10^{-7}) = 1.3 \times 10^{-11}$, in which K_B is assumed $\cong K_A$ (determined by titration) and K_2 is estimated by extrapolation of a Hammett plot of indole pK values.[21] Thus, $k_3 = 5.2 \times 10^{-4}$ s^{-1} (k_3K_1 from Table IV)$/1.3 \times 10^{-11} = 4.0 \times 10^7$ s^{-1}. The effective molarity for the intramolecular general base catalysis is thus the ratio 4.0×10^7 $s^{-1}/4.7 \times 10^{-6}$ M^{-1} $s^{-1} = 8.5 \times 10^{12}$. We can also estimate k_4, the rate constant for uncatalyzed addition of water to C, as $k_4K_2/K_2 = 2.0 \times 10^{-4}$ M^{-1} $s^{-1}/7.9 \times 10^{-7} = 250$ M^{-1} s^{-1}. Thus, catalyzed addition of water to C-2 is $>10^5$ as fast as uncatalyzed addition. This key role for catalysis by carboxylate ion supports our earlier assumption that formation of a tetrahedral intermediate at C-2, rather than its breakdown, is rate-limiting.

For 2-bromoindole-3-acetic acid, intramolecular general catalysis of water addition by the carboxylate group of the zwitterionic intermediate (B) should be even more favorable than with the propionate side chain (six-membered transition state for acetate vs. seven membered for propionate). This expectation is supported by our observation that the hydrolysis of 2-bromoindole-3-acetic acid shows a substantial isotope effect ($k_{H_2O}/k_{D_2O} = 2.0$) in 1 M HCl or DCl (data not shown), while no significant isotope effect is observed with 2-bromoskatole or with **8a** in the same media. Esterification of the side-chain carboxyl group in 2-bromoindole-3-acetic acid reduces the rate constant for hydrolysis by a factor of 400 and a change back to rate-determing water addition, as for 2-bromoskatole. In the case of the acetic acid side chain, therefore, it is clear that water addition has been accelerated to such a degree that proton transfer from H_3O^+ remains rate-limiting even in media as acidic as 1 M HCl (linear k_2 slope in Figure 3). It is also evident from Figure 3 that the value of a presumed k_3K_1 rate for 2-bromoindole-3-acetic acid (and the efficiency of general base catalysis) must be significantly more than 20 times the value for **8a**. Whether the hydrolysis of this compound would exhibit the same effects of pH on mechanism as for **8a** could not be determined readily. At pH values below 0, the hydrolysis of the indoleacetic acid system is too fast to measure without the use of stop flow equipment; at pH values above 1.5, rate plots deviate from linearity and curvature increases with pH, suggesting the formation of a more stable enol lactone intermediate. Initial efforts to detect or trap such an intermediate, however, proved inconclusive.

We can now summarize our interpretation of the mechanisms of hydrolysis of the 2-haloindoles, which mechanisms proved more puzzling and elusive than we might have conceived at the outset:

1. In the absence of a side-chain carboxyl or carboxylate group, water addition at C-2 is always slower than indole protonation (k_4K_2).

2. In the presence of a propionate side chain, water addition is still rate-limiting in the pH region −0.5 to 1.5 (k_3K_1) but has been accelerated by intramolecular general base catalysis; above pH 1.5, such catalysis has become so effective that indole protonation is now rate-limiting (k_2). Above pH 4, intramolecular proton transfer from the carboxyl group (k_1, general acid catalysis)

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(19) Kresge, A. J.; Chen, H. L.; Chiang, Y.; Murrill, E.; Payne, M. A.; Sagatys, D. S. *J. Am. Chem. Soc.* **1971**, *93*, 413–423.
(20) (a) Loudon, G. M.; Ryono, D. E. *J. Am. Chem. Soc.* **1976**, *98*, 1900–1907. (b) Fife, t. H.; Anderson, E. *J. Am. Chem. Soc.* **1971**, *93*, 6610–6614. (c) Buffet, C.; Lamaty, G. *Recl. Trav. Chim. Pays-Bas* **1976**, *95*, 1–6.

is more effective than transfer from external hydronium ion by a factor of 127 M. With respect to the overall enhancement of k_{obsd} above pH 0 (for **3**, **4**, **8**, and **12** relative to 2-haloskatole), intramolecular general base catalysis is much more contributory than general acid catalysis.

3. In the presence of an acetate side chain, water addition to C-2 is even faster and ring protonation is rate-limiting throughout the observable pH range and probably over a much wider range than for propionate.

It is evident from the kinetic data in Tables I and IV that the 2-chloroindoles are consistently more labile to acid hydrolysis than 2-bromoindoles. Rate-limiting hydration at C-2 of the protonated indolenine should be, and is, accelerated by the greater electronegativity of chlorine; when protonation of C-3 becomes rate-limiting, however, the greater electronegativity of chlorine might be expected to reduce the basicity of C-3 and, thus, retard protonation. In fact, the basicity of C-3 is *not* governed by inductive effects alone. Hinman and Lang found 2-alkylindoles significantly more basic than 3-alkylindoles.[9] This phenomenon is readily explained by the fact that the imine or iminium ion produced by C-3 protonation is significantly stabilized by hyperconjugative electron release from the 2-alkyl group.[21] Similarly, a 3p lone pair on chlorine is better able to participate in back-bonding to sp^2 carbon than a 4p lone pair on bromine (compare values of σ_p^+) and the 2-chloroindoles should be the *stronger* bases.

Biological Implications. The facile intramolecular proton transfer from the propionic acid side chain to C-3 of the indole ring suggests that similar processes may occur within the active sites of enzymes or in receptor sites. Thus, a protein carboxyl group may promote indolenine formation during or subsequent to binding of the indole substrate.[22] Furthermore, there is strong reason to believe that the indolenine tautomer of tryptophan is an intermediate in the biosynthesis and catabolism of L-tryptophan, reactions catalyzed by tryptophan synthase and tryptophanase, respectively.[23] Indeed, we have recently obtained experimental support for the presence of the indolenine tautomer in the binding sites of both these enzymes, based on the potent inhibitory properties of oxindolyl-L-alanine and 2,3-dihydro-L-tryptophan;[4] these analogues resemble the indolenine tautomer in having tetrahedral geometry at C-3 of the heterocyclic ring. Furthermore, the two enzymes show opposing specificity for the C-3 diastereoisomers of these analogues.[24]

Just as we have previously demonstrated (via test-tube models) that enzymes can activate substrates considerably by binding highly unfavorable conformations,[25] we now show that activation can also occur by binding a highly unfavorable tautomer (*tenutautomer*). There are many other metabolic species that exist in solution as the overwhelmingly favored members of tautomeric pairs (phenols, imidazoles, etc.), and there is a natural tendency to assume that the enzyme accepts what the spectroscopist sees; we now believe, however, that the phenomenon observed for tryptophan is not necessarily unique and have undertaken an intensive search for additional cases of tenutautomer binding.

Experimental Section

Materials.[26] *N*-Bromosuccinimide and *N*-chlorosuccinimide were recrystallized from glacial acetic acid; the products were washed thoroughly with cold water and were dried in vacuo prior to use. Ethyl indole-3-acetate was obtained from Research Organics, Inc., and methyl indole-3-propionate (**6**) was the generous gift of Dr. T. Spande of this Institute. α-*N*-(Trifluoroacetyl)-L-tryptophan methyl ester (**1**) was obtained from Sigma Chemical Co. or was prepared by reaction of L-tryptophan methyl ester hydrochloride (Research Organics) with ethyl trifluoroacetate in methanol containing triethylamine.[27] 1-Methyl-DL-tryptophan was obtained from Aldrich Chemical Co. Silica gel for column chromatography (Analtech) was 60-Å pore size, 75–150-μm particle size. 2-Bromo- and 2-chloroskatole were prepared by a literature method;[7] these compounds were purified by chromatography on silica gel in $CHCl_3$/petroleum ether (1:2), followed by crystallization from aqueous ethanol. 2-Bromoindole-3-acetic acid[6] was prepared by saponification of the corresponding ethyl ester with 0.5 M sodium carbonate in 50% methanol, as described below for the propionate ester.

α-*N*-(Trifluoroacetyl)-2-bromo-L-tryptophan Methyl Ester (2a). To a suspension of 0.7 g (2.23 mmol) of α-*N*-(trifluoroacetyl)-L-tryptophan methyl ester (**1**) in 15 mL of CCl_4 was added 3 mg of benzoyl peroxide and 0.4 g (2.25 mmol) of finely crushed, recrystallized N-bromosuccinimide. The mixture was refluxed for 2 h under an argon atmosphere and the solvent was evaporated in vacuo. The solid pink residue was dissolved in 10 mL of ethyl acetate and the solution was diluted with 20 mL of hexane and was filtered to remove precipitated succinimide. The filtrate was concentrated and was chromatographed on 50 g of silica gel in ethyl acetate–hexane (1:2). Fractions containing the brominated product, which elutes prior to residual starting material, were pooled and evaporated to a light yellow oil; the oil crystallized upon trituration with petroleum ether to give 0.73 g (83%) of **2a**. Recrystallization from ethyl acetate–petroleum ether gave colorless rosettes, mp 154.5–155.5 °C: UV (EtOH) λ_{max} 219 (ε 41 000), 282 (9500), 289 (8100) nm; $[\alpha]^{20}_D$ −5.5° (MeOH, *c* 0.69); ^{1}H NMR ($CDCl_3$, 220 MHz) δ 3.35 (d, 2, *J* = 6 Hz, β-CH_2), 3.73 (s, 3, OCH_3), 4.92 (m, 1, *J* = 6, 5,5 Hz, α-CH), 6.93 (br d, 1, *J* = 5.5 Hz, α-NH), 7.1–7.5 (m's, 4, Ar H), 8.23 (s, 1, indole NH); MS (CI, NH_3), *m/z* (%) 412 (95) and 410 (1000) (M + 18), 395 (18) and 393 (18) (M + 1). Anal. Calcd for $C_{14}H_{12}BrF_3N_2O_3$: C, 42.77; H, 3.08; N, 7.13; Br, 20.32. Found: C, 43.00; H, 3.15; N, 7.03; Br, 20.36.

α-*N*-(Trifluoroacetyl)-2-bromo-L-tryptophan (3a). A solution of 1.5 g (3.8 mmol) of **2a** in 25 mL of dioxane was added to a solution containing 120 mg of α-chymotrypsin, 112.5 mL of water, 5 mL of 1 M $NaHCO_3$, and 25 mL of dioxane. The clear reaction mixture was stirred at ambient temperature for 3 h and was evaporated to dryness in vacuo. The residual material was suspended in 50 mL of methanol and was filtered through Celite. The flask and filter were washed with an additional 25 mL of methanol and the combined extracts were evaporated in vacuo. The residue was dissolved in 50 mL of water, the solution was extracted with 30 mL of ethyl acetate, and the aqueous layer was acidified with 5 mL of 1 N HCl. The aqueous solution was then extracted with three 50-mL portions of ethyl acetate, the combined extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. The solid residue was dissolved in a minimal volume of ethyl acetate; the product was precipitated by dilution with petroleum ether and was collected. A second crop was obtained by concentration of the filtrate, to give a total of 1.36 g (94%) of colorless powder, mp 150–152 °C: UV (as Na salt in H_2O) λ_{max} 220 (ε 34 000), 280 (7800) and 288 (6400) nm; $[\alpha]^{20}_D$ +20.0° (Na salt in H_2O, *c* 0.5); ^{1}H NMR (CD_3OD, 220 MHz) δ 3.20 and 3.40 (m's, 2, β-CH), 4.69 (m, 1, α-CH), 7.0–7.6 (m's, 4, Ar H); MS (CI, NH_3), *m/z* (%) 398 (93) and 396 (100) (M + 18), 381 (11) and 379 (12) (M + 1), 299 (93) (M − Br). Anal. Calcd for $C_{13}H_{10}BrF_3N_2O_3$: C, 41.18; H, 2.66; N, 7.39. Found: C, 41.23; H, 2.64; N, 6.92.

2-Bromo-L-tryptophan (4a). To a suspension of 156 mg of **3a** (0.41 mmol) in 12 mL of water was added 62 μL of triethylamine (1.09 equiv). After the compound had dissolved, 1 mg of carboxypeptidase A was added. The reaction mixture was stirred for 30 min at ambient temperature and evaporated to dryness and the residue was extracted with 20 mL of methanol. The methanolic extract was filtered through Celite and the solvent was evaporated in vacuo. The residue was suspended in 20 mL of absolute ethanol and the solvent was evaporated. The residue was dissolved in $CHCl_3$ and filtered, giving 118 mg of beige powder. This product was dissolved in the minimal volume of warm methanol (ca. 5 mL); the solution was filtered through a glass wool plug and was evaporated to dryness. The solid residue was suspended in 10 mL of warm absolute ethanol and filtered to give 84 mg (72%) of ivory plates which darken at 170 °C and decompose at 195–200 °C: UV (H_2O) λ_{max} 218 (ε 32 000), 280 (7100), 288 (5800) nm; $[\alpha]^{20}_D$ +1.2° (H_2O, *c* 0.52); ^{1}H NMR (CD_3OD, 220 MHz) δ 3.14 and 3.45 (m's, 2, β-CH), 3.89 (m, 1, α-CH), 7.0–7.7 (m's, 4, Ar H); MS (CI, NH_3), *m/z* (%) 285 (100) and 283 (100) (M + 1), 203 (100) (M − Br). Anal. Calcd for

(21) An analysis of substituent effects on indole p*K* valves will be reported separately. Note that K_2 has been defined as an *association* constant.

(22) Spectral studies (UV and NMR) failed to reveal any detectable concentration of indolenine tautomer in equilibrium with the heavily preferred indole. See Also: Cohen, L. A.; Daly, J.; Kny, H.; Witkop, B. *J. Am. Chem. Soc.* **1960**, *82*, 2184–2187.

(23) Davis, L.; Metzler, D. E. In "The Enzymes"; Boyer, P. D., Ed.: Academic Press: New York, 1972; Vol. VII, pp 33–74.

(24) Phillips, R. S.; Miles, E. W.; Cohen, L. A. *J. Biol. Chem.* **1985**, *260*, 14665–14670.

(25) For example, see: Milstien, S.; Cohen, L. A. *J. Am. Chem. Soc.* **1972**, *94*, 9158–9165.

(26) Microanalyses and mass spectra measurements were performed by the Microanalytical Services and Instrumentation Section of this laboratory, under the direction of Dr. D. F. Johnson. Melting points are uncorrected. The identity and homogeneity of each synthetic compound and of kinetic products were confirmed by MS, NMR, TLC, and HPLC.

(27) Curphey, T. J. *J. Org. Chem.* **1979**, *44*, 2805–2807.

$C_{11}H_{11}BrN_2O_2 \cdot {}^5/_4H_2O$: C, 43.23; H, 4.04; N, 9.17. Found: C, 43.52; H, 3.91; N, 8.91.

α-*N*-(Trifluoroacetyl)-2-chloro-L-tryptophan Methyl Ester (2b). To a suspension of 0.7 g (2.23 mmol) of **1** in 15 mL of CCl_4 was added 3 mg of benzoyl peroxide and 0.3 g (2.25 mmol) of finely crushed, recrystallized *N*-chlorosuccinimide. The suspension was warmed until the protected amino acid dissolved and the mixture was then stirred at ca. 40 °C for 2 h under argon. Workup was exactly as described for the corresponding bromo compound to give 0.50 g (64%) of a pale yellow solid.[28] Recrystallization from ethyl acetate–petroleum ether gave light yellow clusters, mp 150–152 °C: UV (EtOH) λ_{max} 218 (ϵ 44 000), 272 (9900), 289 (7700) nm; $[\alpha]^{20}_D$ −4.3° (MeOH, *c* 0.63); ^{1}H NMR ($CDCl_3$, 220 MHz) δ 3.37 (t, 2, J = 5 Hz, β-CH_2), 3.73 (s, 3, OCH_3), 4.92 (m, 1, α-(CH), 6.93 (br d, 1, α-NH), 7.2–795 (m's, 4, Ar H), 8.20 (s, 1, indole NH); MS (CI, NH_3), *m/z* (%) 368 (36) and 366 (100) (M + 18), 351 (16) and 249 (48) (M + 1). Anal. Calcd for $C_{14}H_{12}ClF_3N_2O_3$: C, 48.21; H, 3.47; Cl, 10.16; N, 8.03. Found: C, 48.22; H, 3.56; Cl, 9.94; N, 8.18.

α-(Trifluoroacetyl)-2-chloro-L-tryptophan (3b). Compound **2b** (0.30 g, 0.86 mmol) was treated with chymotrypsin in the same manner as described for the corresponding bromo compound, yielding 0.23 g (80%) of an amorphous, colorless powder, mp 148–150 °C: UV (Na salt in H_2O) λ_{max} 219 (ϵ 39 000), 272 (8900), 288 (6800) nm; $[\alpha]^{20}_D$ +27° (H_2O + 1.5 equiv of $NaHCO_3$, *c* 0.52); ^{1}H NMR (CD_3OD, 220 MHz) δ 3.3 and 3.4 (m's, 2, β-CH), 4.72 (m, 1, α-CH), 7.0–7.6 (m's, 4, Ar H); MS (CI, NH_3), *m/z* (%) 354 (2) and 352 (6) (M + 18), 337 (1.5) and 335 (5) (M + 1), 299 (18) (M − Cl). An analytical sample was recrystallized from MeOH–H_2O. Anal. Calcd for $C_{13}H_{10}ClF_3N_2O_3 \cdot {}^1/_2H_2O$: C, 45.39; H, 3.20; N, 8.15. Found: C, 45.60; H, 3.31; N, 8.70.

2-Chloro-L-tryptophan (4b). Compound **3b** (0.16 g, 0.48 mmol) was treated with carboxypeptidase as described for the corresponding bromo compound. Workup gave 87 mg (76%) of off-white plates, mp ca 200 °C dec: UV (H_2O) λ_{max} 218 (ϵ 35 000), 271 (7600), 288 (5500) nm; $[\alpha]^{20}_D$ +0.9° (H_2O, *c* 0.55); ^{1}H NMR (CD_3OD, 220 MHz) δ 3.17 and 3.47 (m's, 2, β-CH), 3.97 (d, 1, α-CH, 7.1–7.6 (m's, 4, Ar H); MS (CI, NH_3), *m/z* (%) 241 (30) and 239 (100) (M + 1), 203 (20) (M − Cl), Anal. Calcd for $C_{11}H_{11}ClN_2O_2 \cdot {}^1/_4H_2O$; C, 54.33; H, 4.77; N, 11.52. Found: C, 54.21; H, 4.43; N, 11.94.

Methyl-2-Bromoindole-3-propionate (7a). Methyl indole-3-propionate (**6**, 0.50 g, 2.46 mmol) was reacted with 0.44 g (2.47 mmol) of *N*-bromosuccinimide as described for **1**. The product was obtained as a colorless oil (0.50 g, 72%) after chromatography on silica gel (ethyl acetate–hexane, 1:2). This compound is much less stable than **2a** and darkens rapidly at ambient temperature; UV (MeOH) λ_{max} 221 (ϵ 36 000), 282 (8700), 290 (7300) nm; ^{1}H NMR ($CDCl_3$, 220 MHz) δ 2.64 and 3.06 (t's, 4, J = 7 Hz, α- and β-CH_2's), 3.67 (s, 3, OCH_3), 7.0–7.6 (m, 4, Ar H), 8.18 (s, 1, indole NH); MS (CI, NH_3), *m/z* (%) 301 (95) and 299 (95) (M + 18), 384 (98) and 382 (100) (M + 1).

2-Bromoindole-3-propionic Acid (8a). Compound **7a** (0.27 g, 0.96 mmol) was dissolved in 6 mL of methanol and the solution was diluted with 6 mL of 1 M Na_2CO_3 (the base precipitates). The mixture was heated at gentle reflux for 30 min and was cooled, diluted with water, and extracted with two 20-mL portions of ethyl acetate. The aqueous layer was then acidified to pH 2 and was extracted immediately with two 20-mL portions of ethyl acetate. The latter extracts were dried (Na_2SO_4) and evaporated in vacuo. The residual material was crystallized from ethyl acetate–petroleum ether to give 0.18 g (68%) of off-white clusters, mp 96–98 °C dec: UV (Na salt in H_2O) λ_{max} 280 (ϵ 7100), 222 (30 000) nm; NMR (CD_3OD, 220 MHz) δ 2.56 and 3.00 (t's, 4, J = 7 Hz, α- and β-CH_2), 6.9–7.5 (m, 4, Ar H); MS (CI, NH_3), *m/z* (%) 270 (88) and 268 (90) (M + 1), 210 (98) and 208 (100) (M − CH_2COOH), 188 (85) (M − Br). Anal. Calcd for $C_{11}H_{10}BrNO_2$: C, 49.28; H, 3.76; N, 5.22. Found: C, 49.14; H, 3.98; N, 5.11.

Methyl 2-Chloroindole-3-propionate (7b). Compound **6** (0.6 g, 2.95 mmol) was chlorinated by reaction with 0.40 g of *N*-chlorosuccinimide as described for **1**. The product was obtained as a light yellow oil (0.5 g, 71%); UV (MeOH) λ_{max} 219 (ϵ 41 000), 272 (9700), 289 (5600) nm; ^{1}H NMR ($CDCl_3$, 220 MHz) δ 2.64 and 3.07 (t's, 4, J = 7 Hz, α- and β-CH_2), 3.66 (s, 3, OCH_3), 7.0–7.5 (m, 4, Ar H's); MS (CI, NH_3), *m/z* (%) 239 (10) and 237 (30) (M + 1), 202 (15) (M − Cl).

2-Chloroindole-3-propionic Acid (8b). Compound **7b** (0.12 g, 0.5 mmol) was dissolved in 3 mL of methanol and 3 mL of 1 M Na_2CO_3 was added. The mixture was heated on steam for 30 min and was worked up as described for **8a**. The product was obtained as an oil, which crystallized from ethyl acetate–petroleum ether as off-white needles, mp 89–90 °C (72 mg, 65%): UV (Na salt in H_2O) λ_{max} 220 (ϵ 31 000), 272 (7100) nm; ^{1}H NMR (CD_3OD, 220 MHz) δ 2.56 and 3.02 (t's, 4, J = 7 Hz, α- and β-CH_2), 6.8–7.5 (m, 4, Ar H); MS (CI, NH_3), *m/z* (%) 243 (8) and 241 (20) (M + 18), 226 (20) and 224 (55) (M + 1), 188 (100) (M − Cl). Anal. Calcd for $C_{11}H_{10}ClNO_3$: C, 59.07; H, 4.51; N, 6.26. Found: C, 59.11; H, 4.87; N, 6.30.

α-*N*-(Trifluoroacetyl)-1-methyl-2-bromo-DL-tryptophan Methyl Ester (11a). α-*N*-(Trifluoroacetyl)-1-methyl-DL-tryptophan methyl ester (**10**) was prepared in ca. 40% overall yield by esterification of 1-methyl-DL-tryptophan (Aldrich) in methanol with *p*-toluenesulfonyl chloride,[29] followed by N-trifluoroacetylation with ethyl trifluoroacetate in methanol containing triethylamine.[27] The product (0.25 g, 0.76 mmol) was reacted with 135 mg of *N*-bromosuccinimde as described in **1**. After reaction, the CCl_4 solution was filtered and evaporated in vacuo. The residual material was dissolved in ethyl acetate and the solution was applied to a 20 × 20 cm, 1500-μm silica gel TLC plate. The plate was developed with ethyl acetate–hexane (1:2); the fast-moving material (R_f 0.65) was eluted with ethyl acetate and the extract was evaporated. The crystalline residue was recrystallized from ethyl acetate–petroleum ether to give 0.21 g (68%) of colorless needles, mp 128–129 °C: UV (MeOH) λ_{max} 221 (ϵ 40 000), 283 (11 000), 292 (9200) nm; ^{1}H NMR ($CDCl_3$, 220 MHz) δ 3.41 (d, 2, J = 5 Hz, β-CH_2), 3.75 and 3.77 (s's, 6, 1-CH_3 and OCH_3), 4.94 (m, 1, α-CH), 6.93 (d, 1, α-NH), 7.0–7.5 (m, 4, Ar H); MS (CI, NH_3), *m/z* (%) 426 (75) and 424 (85) (M + 18), 409 (100) and 407 (100) (M + 1), 224 (20) and 222 (20) (M − $CF_3CONHCHCO_2CH_3$). Anal. Calcd for $C_{15}H_{14}BrF_3N_2O_3$: C, 44.24; H, 3.47; N, 6.88; Br, 19.62. Found: C, 44.04; H, 3.74; N, 7.01; Br, 19.91.

α-*N*-(Trifluoroacetyl)-1-methyl-2-bromo-DL-tryptophan (12a). Compound **11a** (61 mg, 0.15 mmol) was dissolved in 2 mL of methanol and 0.16 mL of 1 N NaOH was added. The solution was stirred at ambient temperature for 6 h and was then diluted with water, and the alkaline solution was extracted with two 10-mL portions of ethyl acetate. The aqueous layer was acidified with 0.16 mL of 1 N HCl and was extracted with two 10-mL portions of ethyl acetate. The extracts were dried (Na_2SO_4) and evaporated, to give 41 mg (68%) of an amorphous solid. The solid was dissolved in a small volume of ethyl acetate and the solution was diluted with petroleum ether to give **12a** (31 mg) as a fluffy, colorless solid; UV (Na salt in H_2O) λ_{max} 223 (ϵ 33 000), 283 (7800), 291 (7200) nm; MS (CI, NH_3), *m/z* (%) 395 (90) and 393 (100) (M + 1).

Kinetic Measurements. The hydrolysis of 2-haloindoles can be followed conveniently by spectrophotometry at 280 nm, since the product oxindoles have much lower absorbance at this wavelength ($\Delta\epsilon$ 6000, see Figure 1). Our kinetic reactions were run at 25.0 ± 0.1 °C in a Beckmann DU-8B UV/vis spectrophotometer equipped with a six-cell sample changer and electronic temperature controller. Absorbance values were recorded at convenient intervals (1 min to 1 h), and pseudo-first-order rate constants were obtained from the raw data by use of standard logarithmic plots; if the runs had not gone to completion, the method of Guggenheim[30] was used. In those cases in which both methods were used, identical rate constants were obtained. Reactions were initiated by addition of 15 μL of a 10 mM solution of haloindole (in ethanol, water, or aqueous bicarbonate) to 3 mL of a perchloric acid or acetate buffer medium, which had been allowed to reach temperature equilibrium. In some experiments, especially those carried out at pH 3 or higher, a short lag period (15–30 min) was observed; however, the subsequent reaction followed simple first-order kinetics. Final spectra were identical with those of equivalent concentrations of the authentic oxindoles. In order to avoid the risk of acid hydrolysis, kinetics runs with carboxylic esters were not performed in media stronger than 1 M acid; calculations were based on initial rates although rate plots showed no significant curvature.

Strong acid media were prepared by dilution of 60% $HClO_4$ to desired concentrations and the resulting solutions were standardized to the phenolphthalein end point. Weaker acid media (pH 1–3) were also prepared from $HClO_4$ but were maintained at constant ionic strength (μ = 0.5 M) with $NaClO_4$. For the pH range 3–5, mixtures of 0.1 M acetate buffer and $NaClO_4$ (μ = 0.5 M) were used. pH values were determined at a standard electrode after reactions had reached completion. Buffer catalysis in the acetate buffer media is negligible at 0.1 M concentration, as shown by the fact that identical rates are obtained at pH 3 in either 0.1 M acetate buffer or 1 mM perchloric acid.

Kinetic parameters (k_H and k_{int}, Table I) were determined by regression analysis of plots of k_{obsd} vs. proton activity (h_0)[31] as shown in Figure 2. Values of k_{obsd} for the pH range −1.5 to 5.5 were fitted to eq 1 by use of the MLAB program on the NIH DEC-10 computer system[14] A typical comparison of the calculated curve with experimental data is shown in Figure 3. Isotope effects were evaluated from kinetic measurements (Table II) in media containing D_2O (99.8 atom %) DCl, and

(28) Reflux of the reaction mixture, as in the preparation of **2a**, resulted in significantly lower yields.

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$NaClO_4$ (μ = 0.5); the final deuterium content was >99% in each case. Values of pD were calculated as pH (measured) +0.4.[32]

Semicarbazide Trapping Experiments (Table III). Reaction mixtures contained, in a total volume of 3 mL, 0.6 mmol of NaCl or semicarbazide hydrochloride; pH was adjusted with HCl or KOH, except for the run at pH 3.61, which contained 0.3 mmol of acetate buffer. Reactions were initiated by the addition of 0.5 mL of a 0.3 mM solution of the sodium salt of **8a**. Oxindole product was determined by HPLC analysis on a Rainin C_{18} reverse-phase column (100 × 4.6 mm), with 0.02 M potassium phosphate (pH 7.0)-methanol (4:1) as eluant. Percent oxindole was determined by comparison of peak height in the control reaction (without semicarbazide) to that with semicarbazide. In another control experiment, it was shown that oxindole-3-propionic acid (**9**) does not react with semicarbazide under the experimental conditions.

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Registry No. 1, 1604-49-5; **2a**, 89311-48-8; **2b**, 89311-49-9; **3a**, 89311-50-2; **3b**, 89311-51-3; **4a**, 89311-52-4; **4b**, 89311-53-5; **5**, 32999-55-6; **6**, 5548-09-4; **7a**, 89311-54-6; **7b**, 100572-63-2; **8a**, 100572-64-3; **8b**, 100572-65-4; **9**, 2971-17-7; **10**, 100572-66-5; **11a**, 100572-67-6; **12a**, 100572-68-7; 1-methyl-DL-tryptophane, 26988-72-7; ethyl trifluoroacetate, 383-63-1; 2-bromoskatole, 1484-28-2; ethyl 2-bromoindole-3-acetate, 1912-37-4; 2-bromoindole-3-acetic acid, 1912-39-6; 2-chloroskatole, 51206-73-6.

Regiospecific Sulfonation onto C-3 Hydroxyls of β-Cyclodextrin. Preparation and Enzyme-Based Structural Assignment of 3A,3C and 3A,3D Disulfonates

Kahee Fujita,*[1] Tsutomu Tahara,[2] Taiji Imoto,[1] and Toshitaka Koga[2]

Contribution from the Faculty of Pharmaceutical Sciences, Kyushu University 62, Maidashi, Higashi-ku, Fukuoka 812, Japan, and Daiichi College of Pharmaceutical Sciences, Tamagawa, Minami-ku, Fukuoka 815, Japan. *Received October 7, 1985*

Abstract: A secondary hydroxyl (C-3-OH) of β-cyclodextrin was specifically sulfonated by β-naphthalenesulfonyl chloride in aqueous CH_3CN. This method also gave a limited mixture of the disulfonated β-cyclodextrins, which were mainly composed of 3A,3C and 3A,3D isomers. They were easily isolated by reversed-phase column chromatography and converted to the corresponding diallo epoxides. Their regiochemistries were determined by their specific amylolyses to the linear oligosaccharides.

Chemical construction of enzyme mimics by use of cyclodextrins has attracted much attention.[3] Usually, hydroxyl groups of cyclodextrins are once activated (sulfonated) for the respective functionalizations. Many studies have been focused on the sulfonations of the primary hydroxyl groups since sulfonyl chlorides react exclusively with the primary hydroxyls in pyridine and since the sulfonated cyclodextrins are stable under the sulfonation condition and also under the isolation condition.[4] Activations on secondary hydroxyl groups of cyclodextrins should also be investigated for the sake of wide development of chemical construction of enzyme mimics. Breslow reported selective preparation of 2-deoxy-2-[(*p*-tosyl)oxy]-β-cyclodextrin,[5] and Hattori described formation of 2-deoxy-2-[(*p*-tosyl)oxy]-α-cyclodextrin.[6] Recently, we showed specific preparation of 2-deoxy-2-(*m*-nitrobenzenesulfonyl)oxy]-α-cyclodextrin by the reaction of α-cyclodextrin with the corresponding sulfonyl chloride in an alkaline water.[7] In this report, we describe specific preparation of C-3 sulfonated β-cyclodextrin and its application to specific preparation of 3A,3C- and 3A,3D-disulfonated β-cyclodextrins.

Results and Discussion

β-Cyclodextrin was sulfonated with β-naphthalenesufonyl chloride in 30% aqueous CH_3CN at 40 °C to give a mixture of the C-3 sulfonate **1** and the disulfonates **2** and **3** under the reaction condition where the initial pH of the solution of β-cyclodextrin in the aqueous CH_3CN was adjusted to 12, and the pH was allowed to decrease during the reaction with β-naphthalenesulfonyl chloride. The reaction mixture was not a solution but a suspension of the sulfonyl chloride. The mixture was chromatographed by use of a reversed-phase column to afford pure sulfonates **1** (18.0%), **2** (4.4%), and **3** (4.5%). Structural assignments of these compounds are as follows. The similarity of ^{13}C NMR spectrum of **1** (Figure 1) to that of 3-deoxy-3-[(*p*-tosyl)oxy]-α-cyclodextrin[8] indicated that **1** was the C-3 sulfonate of β-cyclodextrin. Its ^1H NMR and FABMS spectra also confirmed the monosulfonation in **1**. In order to ascertain the C-3 sulfonation, **1** was converted to the allo epoxide **4**, whose structure was spectrally determined as shown below. Its FABMS spectrum showed the correct molecular ion, and its ^{13}C NMR spectrum (Figure 2) demonstrated the presence of the epoxide carbons at δ 58.9 and 56.5. A doublet absorption of one proton (J = 3.30 Hz) was observed at δ 5.05 in its ^1H NMR spectrum (270 MHz). These facts demonstrate that **4** was the allo epoxide,[8] and, therefore, its precursor **1** was the C-3 sulfonate. FABMS and ^1H NMR spectra (Figure 1) showed that **2** and **3** were disulfonates of β-cyclodextrin. Since the present sulfonation reaction gave selectively one product (**1**) as the monosulfonate, the positions of the disulfonation in **2** and **3** were expected to be the C-3 hydroxyls. This expectation was supported by their ^{13}C NMR spectra (Figure 1) where the chemical shifts of the cyclodextrin parts were very similar to that

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