solvent, and the extracts were combined, dried over anhydrous sodium sulfate, filtered, and evaporated to yield the crude isocyanate, which was purified by distillation. The following isocyanates were prepared by this procedure: (a) *n*-heptyl[13] (63%), bp 83–84° (23 mm), $n^{26}D$ 1.4326; (b) *n*-undecyl[13] (54% from petroleum ether, 75% from carbon tetrachloride), bp 144° (18 mm), $n^{26}D$ 1.4358; (c) 9-decenyl[13] (70%), bp 127–128° (17 mm), $n^{26}D$ 1.4446; (d) phenyl (20%), identified by gpc and infrared spectroscopy.

Registry No.—I, 20633-41-4; $C_6H_5NHCONH_2$, 64-10-8; $C_6H_5NHCON(C_2H_5)_2$, 1014-72-8; $C_6H_5NHCONH$-C_4H_9-*n*, 3083-88-3; $C_6H_5NHCON(CH_3)_2$, 101-42-8; $C_6H_5NHCONHCH_2CH_2OH$, 3747-47-5; *n*-$C_7H_{15}NHCONHC_4H_9$-*n*, 20633-46-9; *p*-$ClC_6H_4NHCONHC_4H_7$-*n*, 6333-41-1; *p*-$ClC_6H_4NHCON(C_2H_5)_2$, 15737-37-8; *n*-$C_{11}H_{23}NHCONHCH_3$, 20633-56-1; *n*-$C_{17}H_{35}NHCONHCH_3$, 20633-49-2; $CH_2{=}CH{=}(CH_2)_8NHCONHCH_3$, 20633-50-5; $[(CH_2)_2NHCONHCH_3]_2$, 20633-51-6; $[(CH_2)_3NHCONHCH_3]_2$, 20633-52-7; $[(CH_2)_3NHCONHC_4H_9$-$n]_2$, 20633-53-8; II, 20633-55-0; $[C_6H_5NHCONHCH_2]_2$, 849-97-8; D-gluco-$C_6H_5NHCONHCH_2(CHOH)_4CH_2OH$, 20642-67-5; phenyl carbanilate, 4930-03-4; *n*-$C_7H_{15}NHCOOC_6H_5$, 2594-41-4; 1,1-diallyl-3-isopropylideneurea, 20642-56-2.

(13) V. E. Shoshoua, W. Sweeney, and R. F. Trietz, *J. Amer. Chem. Soc.*, **82**, 866 (1960).

The Effect of the Imidazole Group on the Hydrolysis of N-[2-(4-Imidazolyl)ethyl]phthalimide[1a]

S. C. K. SU AND J. A. SHAFER[1b]

Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48104

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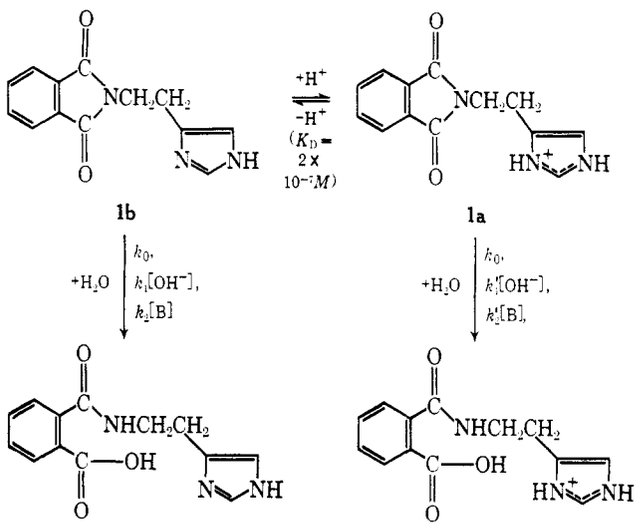
The pH dependencies (at 25°) of the pseudo-first-order rate constants for the hydrolysis (to phthalamic acids) of N-[2-(4-imidazolyl)ethyl]phthalimide (**1**), N-(2-trimethylaminoethyl)phthalimido bromide (**2**), N-(3-trimethylaminopropyl)phthalimido bromide (**3**), and N-methylphthalimide (**4**) were determined. The cationic imides **2** and **3** are most susceptible to hydroxide ion catalyzed hydrolysis. Below pH 7, however, **1** hydrolyzes most rapidly. This effect was ascribed to the neighboring imidazole residue functioning as a general base in catalyzing attack by water. A deuterium oxide solvent isotope effect of 2.1 is associated with this process. The possibility that this effect reflects the susceptibility of the protonated form of **1** to attack by hydroxide ion was deemed unlikely, since the second-order rate constant for this reaction would be 333 $sec^{-1}\,M^{-1}$, while the second-order rate constants for the hydroxide ion catalyzed hydrolysis of cationic imides **2** and **3** with their positive charges closer to the carbonyl carbon atom are 91 and 41 $sec^{-1}\,M^{-1}$, respectively. Also, cationic imides **2** and **3** are susceptible to direct attack by water, whereas the protonated form of **1** is much less susceptible to attack by water. It is unlikely that a neighboring imidazole residue functions as a general acid in catalyzing attack by hydroxide ion, since the calculated rate constant for this process is not significantly lowered by deuterium oxide. The first-order rate constants for intramolecular catalysis of the hydrolysis of **1** by the neighboring imidazole group (2.9×10^{-5} sec^{-1}) was found to be similar in magnitude to the second-order rate constant for the imidazole-catalyzed hydrolysis of **4** (2.0×10^{-5} $sec^{-1}\,M^{-1}$).

Neighboring amide groups are potent nucleophiles, and under physiological conditions amide groups can enhance the rate of hydrolysis of adjacent ester and amide residues by several orders of magnitude.[2] Often, the rate-limiting step in these reactions is the hydrolysis of the imide intermediate. Because of the possible importance of the amide group in enzymically catalyzed hydrolytic reactions, we have investigated the effect of the imidazole group on the hydrolysis of N-[2-(4-imidazolyl)ethyl]phthalimide (**1**) (Scheme I).

SCHEME I



Experimental Section

Materials.—N-Methylphthalimide was obtained from Eastman Organic Chemicals and recrystallized twice from 95% ethanol: mp 134–135° cor (lit.[3] mp 133–134°).

N-(2-Trimethylaminoethyl)phthalimido bromide was prepared by mixing 5.08 g (20 mmol) of N-(2-bromoethyl)phthalimide (from Eastman Organic Chemicals) dissolved in 75 ml of dioxane with 5.4 g (91 mmol) of trimethylamine (from Eastman Organic Chemicals) dissolved in 150 ml of dioxane. After the mixture stood overnight at room temperature, the precipitate was collected and recrystallized three times from 95% ethanol: dec pt 290–291° cor. *Anal.* Calcd for $C_{13}H_{17}N_2O_2Br$: C, 49.85; H, 5.47; N, 8.95; Br, 25.52. Found: C, 49.59; H, 5.46; N, 8.75; Br, 25.20.

N-(3-Bromopropyl)phthalimide was prepared from potassium phthalimide (from Eastman Organic Chemicals) and 1,3-dibromopropane (from CalBiochem) according to the method of Gabriel:[4] mp 71–73° cor (lit.[4] mp 72–73°).

N-(3-Trimethylaminopropyl)phthalimido bromide was prepared by mixing 2.68 g (10 mmol) of N-(3-bromopropyl)phthal-

(1) (a) This study was supported by a grant (AM-09276) from the National Institutes of Health, U. S. Public Health Service. (b) To whom inquiries regarding this work should be made.

(2) S. C. K. Su and J. A. Shafer, *J. Org. Chem.*, in press, and references therein.

(3) M. Freund and H. Beck, *Ber.*, **37**, 1942 (1904).

(4) S. Gabriel and J. Weiner, *ibid.*, **21**, 2669 (1888).

imide dissolved in 15 ml of dioxane with 1.65 g (28 mmol) of trimethylamine dissolved in 25 ml of dioxane. After the mixture stirred overnight at room temperature, the precipitate was collected and recrystallized once from 95% ethanol and twice from 2-propanol: dec pt 209–211° cor. *Anal.* Calcd for $C_{14}H_{19}N_2O_2Br$: C, 51.38; H, 5.85; N, 8.56; Br, 24.42. Found: C, 51.22; H, 6.15; N, 8.51; Br, 24.21.

N-[2-(4-Imidazolyl)ethyl]phthalimide was prepared by refluxing 2.96 g (20 mmol) of phthalic anhydride (from Eastman Organic Chemicals) with 2.22 g (20 mmol) of histamine (from CalBiochem) in 200 ml of glacial acetic acid. After 30 min, 150–160 ml of acetic acid was distilled from the reaction flask and the remaining solution was cooled and poured into 100 ml of ether. The resulting precipitate was dried at 100° under reduced pressure (to convert any acetate salt of the product into the free base) and then recrystallized three times from benzene and heated at 100° for 48 hr under reduced pressure: mp 189–190° cor. *Anal.* Calcd for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.72; H, 4.55; N, 17.35.

Imidazole was obtained from the Aldrich Chemical Co., recrystallized three times from benzene and sublimed under reduced pressure: mp 88–89 cor (lit.[5] mp 90.2–90.6°).

Tris(hydroxymethyl)aminomethane (Tris, ultrapure) was obtained from Mann Research Laboratories.

Deuterium oxide and DCl in deuterium oxide were obtained from Biorad Laboratories.

The distilled water supplied to the laboratory was run through a demineralizer and redistilled in an all-glass still.

All other chemicals used were Mallinckrodt or Baker–Adamson analytical reagents.

Methods.—Measurements of pH and pD were made using a Radiometer Model 4b pH meter which was standardized with a 1:1 phosphate, NBS primary standard solution.[6] The response of the glass electrode was checked with another NBS primary standard solution (either borax or phthalate). Any nonideality in the glass electrode response was corrected with the temperature compensator. This correction never corresponded to more than 1°/pH unit difference between the primary standards. Measurements of pH and pD were made before and after each kinetic run, and the average value of pH was used. The total change in pH during a kinetic run in a buffered solution rarely exceeded 0.02 unit.

Hydrogen ion concentrations were estimated from the pH and the mean activity coefficient of hydrogen chloride in potassium chloride solutions. The mean activity coefficient used (0.75 at $\Gamma/2 = 0.20\ M$) was interpolated from the data listed by Harned and Owen.[7] The hydroxide ion concentration was estimated from the hydrogen ion concentration and the formal dissociation constant for water (K_W'), *i.e.*, $K_W\alpha_{H_2O}/\gamma_{H^+}\gamma_{OH^-}$ which was interpolated from data listed in ref 7. The formal dissociation constant used was $1.74 \times 10^{-14}\ M$ at $\Gamma/2 = 0.20\ M$.[8]

Rate Measurements.—The disappearance of imides was followed spectrophotometrically at 300 mμ using a Gilford Model 2000 multiple-sample absorbance recorder.

Reactions in buffered solutions were carried out in a cuvette equipped with a ground-glass stopper. The buffers used were KH_2PO_4–Na_2HPO_4, Tris HCl, and $NaHCO_3$–Na_2CO_3. The absorbance was monitored continuously, and the temperature was controlled (25° ± 0.05°) by circulating water from a thermostated bath through the thermospacers surrounding the cell compartment. The temperature of the reacting solution was determined with a NBS certified thermometer. The approach of the absorbance (A) to its final value (A_∞) was first order. Pseudo-first-order rate constants (k_{obsd}) were determined by the method of Guggenheim[9] or from the slopes of the linear plots of $-\ln(A - A_\infty)$ *vs.* time. The independence of the rate constants on the initial concentration of imide also established that the reaction was first order in imide.

Below pH values of 7.5, the hyrolysis of imide to amic acid does not go to completion,[2] and the concentration of imide in equilibrium with amic acid becomes significant. Therefore, below pH values of 7.5, k_{obsd} was determined from the initial rate of disappearance of imide (R) using relationship 1. The initial

$$R = -\frac{1}{E} \times \frac{dA}{dt} = k_{obsd}[I^0] \quad (1)$$

slope of the absorbance *vs.* time plot at 300 mμ is represented by dA/dt, while $[I^0]$ is the initial concentration of imide, and E is the extinction coefficient of the imide. All initial rates were obtained from measurements made before 6% of the final concentration of N-methylphthalamic acid was reached, and these initial absorbance *vs.* time plots were linear. For initial rate measurements, full scale on the recorder was set to 0.1 absorbance unit. For other measurements of absorbance, full scale on the recorder was set to 1.0 absorbance unit.

The products of the reactions were characterized by the identity of their ultraviolet spectra taken at the end of the kinetic runs with the spectra of the amic acids. When the reaction did not go to completion, the final spectrum could be rationalized by assuming a mixture of imide and amic acid.[10] Ultraviolet spectra were determined on a Cary Model 15 recording spectrophotometer. The formal acid dissociation constant (K_D) for **1a** was determined by dissolving **1** in acid and back titrating with base to pH 6. (Above pH 6, the hydrolysis of **1** interferes with the determination of the titration curve.) Values of K were calculated from the relationship $K_D = [H^+][\mathbf{1b}]/[\mathbf{1a}]$ at several points in the titration curve. The average value of K_D was $2.0 \times 10^{-7}\ M$ in water and $0.62 \times 10^{-7}\ M$ in deuterium oxide.

Results

Equation 2 should describe the observed depencence (Table I) on the hydroxide ion and the buffer ([B]) concentration of the pseudo-first-order rate constant for the hydrolysis of N-[2-(4-imidazolyl)ethyl]phthalimide (**1**), where α is the fraction of imide with an

$$k_{obsd} = \alpha(k_0 + k_1[OH^-] + k_2[B]) + (1 - \alpha)(k_0' + k_1'[OH^-] + k_2'[B]) \quad (2)$$

unprotonated imidazole residue. The two terms on the right side of eq 2 represent the contribution to k_{obsd} made by the hydrolysis of **1b** and **1a**, respectively. Since

$$\alpha = \frac{K_D}{[H^+] + K_D} \quad (3)$$

$$k_{obsd}/\alpha = k_0 + k_1'K_W'/K_D + k_1[OH^-] + k_2[B] + k_0'[H^+]/K_D + k_2'[B][H^+]/K_D \quad (4)$$

The ratio, k_{obsd}/α was evaluated using values of $2.0 \times 10^{-7}\ M$ and $0.62 \times 10^{-7}\ M$ for the formal acid dissociation constant (K_D) of N-[2-(4-imidazolyl)ethyl]phthalimide in water and deuterium oxide (see Experimental Section).

For the concentrations of buffer used, catalysis by buffer accounted for less than 15% of the observed rate. At pH 9.8, the $[OH^-]$-catalyzed reaction makes the predominant contribution to the observed rate constant, and a plot of $k_{obsd}/[OH]\alpha$ *vs.* $[B]/[OH^-]$ was extrapolated to $[B] = 0$ to obtain the value of $k_{obsd}/[OH^-]$ in the absence of buffer.[11] In the pH range of 5.2–8.2, values of k_{obsd}/α in the absence of buffer were determined by extrapolating plots[11] of k_{obsd}/α *vs.* [B] to [B] = 0. The effect of the small changes in $[OH^-]$ accompanying changes in the buffer concentra-

(5) F. Cramer, *Angew. Chem.*, **72**, 236 (1960).

(6) (a) R. G. Bates, "Determination of pH Theory and Practice," John Wiley & Sons, Inc., New York, N. Y., 1964, pp 62–94, 123–130. (b) pD = pH meter reading + 0.40; ref 6a, p 220.

(7) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed, Reinhold Publishing Corp., New York, N. Y., 1958, pp 638, 748, and 752.

(8) The effect of KCl on the activity coefficients in water and deuterium oxide were assumed to be equivalent, so that the value (6.5) given by R. W. Kingerly and V. K. La Mer [*J. Amer. Chem. Soc.*, **63**, 3256 (1941)] for the dissociation constant of water relative to the dissociation constant of deuterium oxide could be used to calculate $[OD^-]$.

(9) E. A. Guggenheim, *Phil. Mag.*, [7] **2**, 538 (1926).

(10) J. Brown, S. C. K. Su, and J. A. Shafer, *J. Amer. Chem. Soc.*, **88**, 4468 (1966).

(11) At a constant ratio of the acidic to basic component of the buffer.

TABLE I

THE EFFECT OF pH AND BUFFERS ON THE HYDROLYSIS OF N-[2-(4-IMIDAZOLYL)ETHYL]PHTHALIMIDE AT 25° [a]

pH	Buffer	Buffer concn, M[b]	$10^5 k_{obsd}$, sec^{-1}	$10^5 k_{obsd}/\alpha$, sec^{-1} Obsd	$10^5 k_{obsd}/\alpha$, sec^{-1} Calcd[c]
5.28[d]	1:5 NaAc–HAc			2.8[e]	2.9
5.26		0.0277	0.157		
5.28		0.0462	0.168		
5.13		0.0924	0.187		
5.90[d]	1:6 Na_2HPO_4–KH_2PO_4			3.0[e]	2.9
5.89		0.0304	0.31		
5.90		0.0608	0.33		
5.91		0.122	0.34		
7.32[d]	4:1 Na_2HPO_4–KH_2PO_4			3.2[e]	3.2
7.28		0.0151	2.4		
7.32		0.0302	2.5		
7.36		0.0604	2.7		
7.57[d]	7:1 Na_2HPO_4–KH_2PO_4			3.3[e]	3.4
7.55		0.0284	3.1		
7.57		0.0428	3.3		
7.60		0.0572	3.3		
8.20[d]	1:1 Tris–Tris HCl			5.2[e]	5.2
8.19		0.0314	5.2		
8.20		0.0628	5.4		
8.20		0.0942	5.5		
9.81[d]	10:11 Na_2CO_3–$NaHCO_3$			97[f]	96
9.78		0.0206	94		
9.82		0.0412	106		
9.84		0.0618	111		

[a] $\Gamma/2 = 0.2$ maintained with KCl. [b] Acidic + basic component. [c] Calculated using the expression $k_{obsd}/\alpha = 2.9 \times 10^{-5} + 11$ [OH$^-$]. [d] Average pH for this buffer. [e] Evaluated by extrapolatng a plot of k_{obsd}/α *vs.* [B] to [B] = 0. [f] Evaluated by extrapolating a plot of $k_{obsd}/$[OH$^-$]α *vs.* [B]/[OH$^-$] to [B] = 0, and using this extrapolated value of $k_{obsd}/$[OH$^-$]α to calculate k_{obsd}/α at pH 9.81.

tion on the value of k_{obsd}/α was not significant, and, for the purposes of extrapolation, plots of k_{obsd}/α *vs.* [B] were assumed to be linear.

The depencence on the hydroxide ion concentration of the values of k_{obsd}/α at [B] = 0 should be described by eq 5.

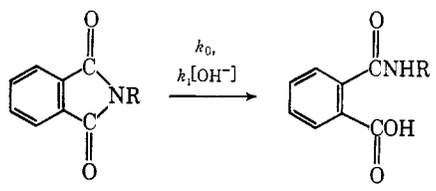
$$k_{obsd}/\alpha = k_0 + k_1K_W'/K_D + k_1[OH^-] + k_0'[H^+]/K_D \quad (5)$$

The data in Table I reveal that the contribution of the term $k_0'[H^+]/K_D$ to k_{obsd}/α is insignificant, since the dependence of k_{obsd}/α on the hydroxide ion concentration (at [B] = 0) may be represented by expression 6 where $k_0 + k_1'K_W'/K_D = 2.9 \times 10^{-5}$ sec^{-1}

$$k_{obsd}/\alpha = k_0 + k_1'K_W'/K_D + k_1[OH^-] \quad (6)$$

and $k_1 = 11$ sec^{-1} M^{-1}. In deuterium oxide, values of 1.4×10^{-5} sec^{-1} and 16 sec^{-1} M^{-1} were observed for $k_0 + k_1'K_W'/K_D$ and k_1, respectively.

Figure 1 compares the pH dependencies of the observed pseudo-first-order rate constants for the hydrolysis of imides **1**, **2**, **3**, and **4**, thereby illustrating the effect of the imidazole group on the hydrolysis of **1**. Values for k_0 and k_1 are listed in Table II.



2, R = $CH_2CH_2N^+(CH_3)_3Br^-$
3, R = $CH_2CH_2CH_2N^+(CH_3)_3Br^-$
4, R = CH_3

TABLE II

RATE CONSTANTS FOR THE HYDROLYSIS OF SOME PHTHALIMIDES TO PHTHALAMIC ACIDS AT 25°

Phthalimide	k_1,[a] sec^{-1} M^{-1}	$10^7 k_0$,[b] sec^{-1}
2	91	3.6
3	41	2.6
4	24	

[a] Obtained by extrapolating plots of $k_{obsd}/$[OH$^-$] *vs.* [buffer]/[OH$^-$] to [buffer] = 0 at pH values above 7. [b] Obtained by extrapolating plots of $k_{obsd} - k_1$[OH$^-$] *vs.* [buffer] to [buffer] = 0 at pH values below 7.

Examination of Table III reveals that the imidazole residue in **1** perturbs the ultraviolet absorption spectrum of the phthalimido group. Interestingly, concentrated solutions of imidazole cause similar perturbations in the ultraviolet spectrum of N-methylphthalimide. These results suggest that the imidazole residue in **1** is interacting with the phthalimido group.

TABLE III

THE EFFECT OF IMIDAZOLE ON THE ABSORBANCE OF SOME PHTHALIMIDES

Phthalimide	Conditions	E_{300}/E_{250}[a]
1	0.025 M KH_2PO_4–0.025 M Na_2HPO_4	2.79[b]
2		5.28
3		5.63
4		5.26
4	0.434 M imidazole–0.434 M imidazole HCl	3.43[b]

[a] E_{250} and E_{300} represent the extinction coefficients of the imides. The extinction coefficients of these imides were independent of the imide concentration. [b] The low value for this ratio is mainly attributable to an increase in E_{250}.

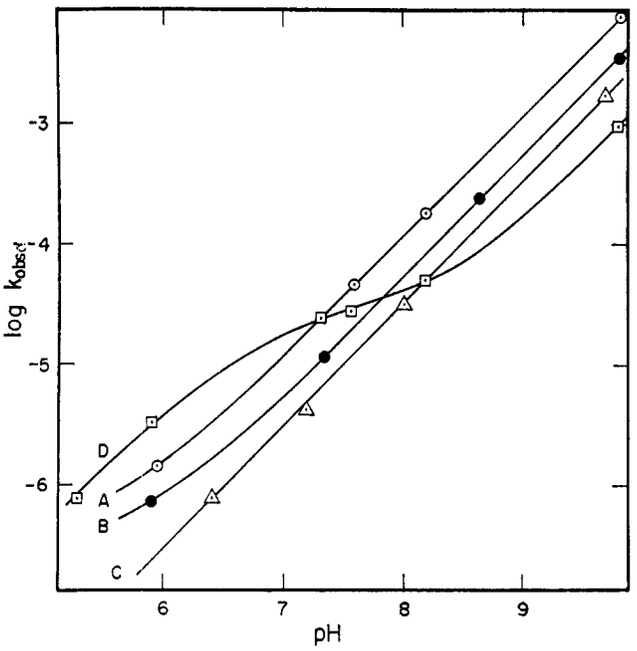


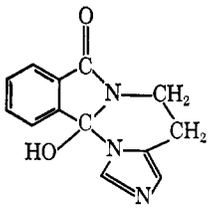
Figure 1.—The pH dependencies of the observed pseudo-first-order rate constant for the hydrolysis of some N-substituted phthalimides to the corresponding phthalamic acids at 25°: A, N-(2-trimethylaminoethyl)phthalimido bromide (2), ⊙ (solid line calculated from $k_{obsd} = 3.6 \times 10^{-7} + 91[OH^-]$); B, N-(3-trimethylaminopropyl)phthalimido bromide (3), ● (solid line calculated from $k_{obsd} = 2.6 \times 10^{-7} + 41[OH^-]$); C, N-methylphthalimide (4), △ (solid line calculated from $k_{obsd} = 24[OH^-]$); D, N-[2-(4-imidazolyl)ethyl]phthalimide (1), □ [solid line calculated from $k_{obsd}/\alpha = 2.9 \times 10^{-5} + 11[OH^-]$ and $\alpha = (2.0 \times 10^{-7})/([H^+] + 2.0 \times 10^{-7})$].

Discussion

It is not surprising that in basic solution the cationic imides 2 and 3 are more susceptible to hydroxide ion catalyzed hydrolysis than imides 1 and 4. Similarly, the enhanced reactivity of 1 at pH values near neutrality might be attributed solely to electrostatic effects associated with the protonated form of 1. In terms of eq 4, this assumption means $k_1'K_W'/K_D \gg k_0$. Therefore, $k_1' = 333$ sec^{-1} M^{-1} (eq 6). It is unlikely, however, that **1a** with its center of positive charge further removed from a carbonyl carbon atom would be more susceptible to hydroxide ion catalyzed hydrolysis than 2 and 3 (Table II). Also, if electrostatic effects were responsible for the enhanced reactivity of 1 in solutions near neutrality, the "spontaneous" or water-catalyzed hydrolysis of **1a** would also be expected to be increased. In reality, the fit of eq 6 to the data in Table I requires that the term representing "spontaneous" hydrolysis of **1a** (k_0') be less than the corresponding terms assigned to 2 and 3.[12] It is therefore more reasonable to assume that the imidazole residue in 1 catalyzes the hydrolysis of the phthalimido group.

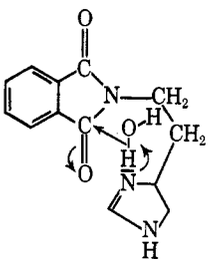
We have previously reported the catalysis and inhibition of the hydrolysis of 4 by imidazole.[2] Spectroscopic and kinetic evidence was presented in that report suggesting that imidazole acts as a general base in catalyzing the hydrolysis of 4, and that imidazole may form an unreactive tetrahedral addition compound with 4.

The increased absorbance of 1 at wavelengths below 300 mμ suggests that the interaction between the imidazole and phthalimido group in 1 is similar to the interaction between imidazole and N-methylphthalimide. Perhaps a fraction of 1 exists as the unreactive tetrahedral addition compound 5. The low value



5

observed for the second-order rate constant for hydroxide ion catalyzed hydrolysis of 1 (11 sec^{-1} M^{-1} *vs.* 24 sec^{-1} M^{-1} for 4) is also consistent with the existence of a substantial amount of 5. Expulsion of amide anion from 5 to form the acylimidazole seems unlikely, since the ability of imidazole to displace groups from a carbonyl carbon atom decreases sharply as the pK_a of the leaving group becomes larger than 10.[13] The observed deuterium isotope effect of 2.1 for k_0 also argues against nucleophilic displacement of an amide anion by a neighboring imidazole group. It is unlikely that imidazolium ion is functioning as a general acid in catalyzing attack by hydroxide ion, since the calculated rate constant for this process is not significantly lowered by deuterium oxide.[14] It therefore seems reasonable to assume that imidazole is functioning as a general base in catalyzing attack by water on imide 1.



6

The first-order rate constant (k_0) for the intramolecularly catalyzed hydrolysis of 1 by imidazole is estimated as 2.9×10^{-5} sec^{-1}, while the second-order rate constant for the imidazole-catalyzed hydrolysis of N-methylphthalimide is 2.0×10^{-5} sec^{-1} M^{-1}. The ratio of these two rate constants, 1.5 M, gives the effective local concentration of imidazole in 6.

This ratio can be compared with the value of 13 M estimated by Ferscht and Kirby[15] for the effective

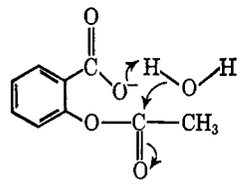
(12) If the value of k_{obsd} were in error by 10% at pH 5.28, then the maximum value of k_0' $[H^+]/K_D$ would be $1.1 \times 2.8 \times 10^{-5} - 2.9 \times 10^{-5}$ and the upper limit of k_0' would be 0.52×10^{-7} sec^{-1}. This value is less than the corresponding values (k_0) assigned to 2 (3.6×10^{-7} sec^{-1}) and 3 (2.6×10^{-7} sec^{-1}).

(13) J. F. Kirsch and W. P. Jencks, *J. Amer. Chem. Soc.*, **86**, 837 (1964).

(14) Assuming that general acid catalysis by the neighboring imidazolium ion is important, $k_1' = [K_D/K_W']_{H_2O} \times 2.9 \times 10^{-5}$ or 333 sec^{-1} M^{-1} in water and $k_1' = [K_D/K_W']_{D_2O} \times 1.4 \times 10^{-5}$ or 324 sec^{-1} M^{-1} in deuterium oxide.

(15) A. R. Ferscht and A. J. Kirby, *ibid.*, **89**, 4857 (1967).

concentration of carboxylate in the hydrolysis of aspirin.



7

A difference in the relative orientation of the catalytic residue and the carbonyl carbon atom in **6** and **7** is probably responsible for the difference in the efficiencies of the two catalysts. Also, if a significant fraction of **1** exists, in the unreactive tetrahedral form **5**, the estimate of the effective concentration of the imidazole residue in **6** would be too low.

Registry No.—**1**, 5959-80-8; **2**, 20452-82-8; **3**, 20452-83-9; **4**, 550-44-7.

Stereochemistry of Flexuosin A and Related Compounds[1,2]

WERNER HERZ, P. S. SUBRAMANIAM, AND N. DENNIS

Department of Chemistry, The Florida State University, Tallahassee, Florida 32306

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2-Acetylflexuosin A and autumnolide, two new sesquiterpene lactones, were isolated from an Alabama collection of *Helenium autumnale* L. Correlation of the former with linifolin A led to the elucidation of the complete stereochemistry of flexuosin A and its congeners.

Helenium autumnale L. collections of unspecified provenance are reported[3] to serve as sources of the pseudoguaianolide helenalin (**1**), but more recent extractions of plant material from North Carolina[4] and Pennsylvania[5] yielded other sesquiterpene lactones and no helenalin.[6] In an effort to shed light on these variations we have examined several southeastern collections of *H. autumnale*. Material from northwestern Florida and southern Georgia gave respectable yields of helenalin, as claimed in the early[3] literature. On the other hand material from Greene County, Ala., gave instead of helenalin two previously unreported sesquiterpene lactones. The study of these compounds allowed us to clarify the structure of flexuosin A[10] and is described in this paper.

The substance obtained in larger yield (0.3%), $C_{19}H_{26}O_7$, mp 124–126°, exhibited ir bands (see Experimental Section) which indicated the presence of one hydroxyl group, two esters, and an α,β-unsaturated γ-lactone. That the ester bands were derived from two secondary acetates as suggested by the empirical formula was verified by the nmr spectrum, which had two singlets at 1.98 and 2.00, a doublet at 5.36, and a triplet of doublets at 4.52 ppm. The nmr spectrum also had the signals characteristic of the usual α,β-unsaturated γ-lactone function associated with pseudoguaianolides of the helenalin series (two narrowly split doublets at 6.18 and 5.97 and a complex triplet at 4.9 ppm), one secondary hydroxyl group (broadened doublet at 3.8 ppm), a methyl singlet, and a methyl doublet.

Acetylation of the new lactone afforded diacetylflexuosin A (**2d** devoid of stereochemistry) of known structure and uncertain configuration.[10] Comparison of the nmr spectra of the new lactone, flexuosin A (gross structure **2a**),[10,11] alternilin (gross structure **2b**),[11] and **2d** gave clear evidence for its formulation as 2-acetylflexuosin A (gross structure **2c**). The broadened doublet associated with H-4 exhibited the same chemical shift in the nmr spectra of **2a**, **2b**, and the new lactone, but moved downfield on acetylation to **2d**. On the other hand, **2d** and the new lactone displayed identical chemical shifts for the triplet of doublets associated with H-2 and the doublet associated with H-6.[12]

Oxidation of the new sesquiterpene lactone gave the cyclopentanone derivative **3** (new carbonyl band at 1750 cm^{-1}) whose nmr spectrum (absence of 3.8-ppm signal) confirmed the assignments of the previous paragraph. Pyrolysis of **3** in a nitrogen atmosphere afforded a crystalline substance identical in all respects with linifolin A, whose absolute configuration has been shown[11] to be **4**. This result defined the previously unknown asymmetric centers C-1, C-5, C-6, C-7, and C-8 of flexuosin A, alternilin, and their congeners.

There remained the problem of determining the configuration at C-2 and C-4. Unlike pulchellin,[13] flexuosin A could not be induced to form a carbonate or a sulfite. Hence, the hydroxyl groups were *trans*. The nature of the five-membered ring is such that this information, even with knowledge of the absolute configuration at C-1 and C-5 and of the coupling constants $J_{H\text{-}1,H\text{-}2}$, $J_{H\text{-}2,H\text{-}3a}$, $J_{H\text{-}2,H\text{-}3b}$, $J_{H\text{-}3a,H\text{-}4}$, and $J_{H\text{-}3b,H\text{-}4}$, does not permit an unambiguous assignment of stereochemistry to C-2 and C-4. However, the configura-

(1) Constituents of *Helenium* Species. XXIII. Previous paper, L. Tsai, R. J. Highet, and W. Herz, *J. Org. Chem.*, **34**, 945 (1969).

(2) Supported in part by grants from the National Science Foundation (GP-6362) and the National Institutes of Health (GM-05814).

(3) For leading references see W. Herz, A. Romo de Vivar, J. Romo, and N. Viswanathan, *J. Amer. Chem. Soc.*, **85**, 19 (1963); W. Herz and P. S. Santhanam, *J. Org. Chem.*, **32**, 507 (1967).

(4) R. A. Lucas, R. G. Smith, and L. Dorfman, *ibid.* **29**, 2101 (1964).

(5) W. Herz and P. S. Subramaniam, unpublished results.

(6) The existence of subspecies or varieties may be responsible for these differences. A collection labeled *H. autumnale* var. *canaliculatum* (Lam.) T. and G. (equivalent to *H. latifolium* Mill. according to Rydberg,[7] but not according to Gleason and Cronquist[8] where *H. latifolium* is absorbed in *H. autumnale* var. *autumnale*) furnished[9] tenulin and no helenalin.

(7) P. A. Rydberg, *North American Flora*, **34**, part 2, 119(1915).

(8) H. A. Gleason and A. Cronquist, "Manual of Vascular Plants of the Northeastern United States and Adjacent Canada," D. Van Nostrand Company, Princeton, N. J., 1963.

(9) Unpublished work by B. F. Aycock and A. E. Senear, cited by R. Adams and W. Herz, *J. Amer. Chem. Soc.*, **71**, 2546 (1949).

(10) W. Herz, Y. Kishida, and M. V. Lakshmikantham, *Tetrahedron*, **20**, 979 (1964).

(11) W. Herz, C. M. Gast, and P. S. Subramaniam, *J. Org. Chem.*, **33**, 2780 (1968).

(12) The isolation of authentic **2c** requires that the monoacetylflexuosin A, mp 158–160°, obtained previously[10] in low yield by treatment of flexuosin A with isopropenyl acetate–toluenesulfonic acid be formulated as **2e** (4-acetylflexuosin A).

(13) W. Herz, K. Ueda, and S. Inayama, *Tetrahedron*, **19**, 483 (1963).