General Base Catalytic Activity of 2-Substituted Imidazoles for Hydrolysis of Ethyl Dichloroacetate

By **Masayasu Akiyama,* Masao Ihjima,** and **Yukihiro Hara,** Department of Industrial Chemistry, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184, Japan

The hydrolysis of ethyl dichloroacetate catalysed by a series of 2-substituted imidazoles has been studied in water at 30°. The substituents are Me, Et, and Pr^{i}, and a few hydroxyalkyl groups. Activation parameters have been determined for some of the imidazoles. Neither general acid nor nucleophilic catalysis has been detected for the reactions, confirming the general base catalysis mechanism. The catalytic activities of the imidazoles are evaluated from the extrapolated rate values derived from the k_{im} values at 30° assuming a common Brönsted slope of 0.47 and an equal pK_a of 7.80. It is found that the sensitivity of the substituents to Taft steric constant parameters is rather small (0.26) and that the hydroxy-group in the substituent acts favourably for the catalysis (by a factor of 1.7). The latter is explained in terms of a microsolvent effect.

In the hydrolysis of carboxylate esters,[1-4] general base catalysis by imidazole has been established by Jencks and his co-workers for esters with poor leaving groups.[5-7] General base catalysis is important for studies of enzyme models, because it is involved in the enzyme hydrolysis process; a histidine imidazolyl group acts as a general base to assist the formation and breakdown of an acyl-serine intermediate.[8] Most studies on imidazole catalyses, however, have dealt with nucleophilic catalysis, and those of general base catalysis are limited to instances [1-4] where alkyl carboxylates have been used as substrates [9-15] Thus, intermolecular general base catalysis by imidazoles is accelerated by a hydroxy-group in the alcohol moiety [9] or by a carboxy-group in the imidazole moiety [10] but retarded by bulky substituents in the acyl portion of esters.[11] Intramolecular catalysis is enhanced by freezing internal rotation [13] or by properly fixing both ester and imidazolyl functions in a rigid norbornane system.[14] In a few special cases, general base catalysis by imidazole has been observed even for aryl esters.[16,17] Since an imidazolyl group of an enzyme active site is in a unique environment where electric, hydrophilic, hydrophobic, and steric effects operate simultaneously, it is of interest to examine the behaviour of alkyl- and hydroxyalkyl-substituted imidazoles as general base catalysts. In addition, there are questions from a previous study [17] on these substituted imidazoles, *e.g.* by how much does steric bulk of the imidazole ring diminish the general base activity and does a hydroxy-group in the substituent facilitate general base catalysis? This paper reports the catalytic activity of 2-substituted imidazoles toward the hydrolysis of ethyl dichloroacetate. The substrate is well known for its susceptibility to catalysis by general bases involving imidazole.[5]

EXPERIMENTAL

Ethyl dichloroacetate was prepared and distilled, b.p. 67—68° at 31 mmHg, n_D^{20} 1.4383 (lit.,[5] b.p. 61—61.5° at 19 mmHg, n_D^{20} 1.438 6). Sources of imidazoles and preparation of buffers were described previously.[17] Water distilled from glass and spectroscopic grade acetonitrile were used.

Conductivity measurements were made in a Toadenpa CG 210 PL cell.[18] A Kohlrausch bridge YKR-2 (Yamabishi Electric Co.) equipped with an amplifier and oscillator and platinum electrodes were used for measurements. A constant temperature was maintained by circulation of water (±0.1 °C).

^{1}H N.m.r. spectra were recorded on a JEOL C-60HL instrument at 25°.

The pK_a values of imidazoles were determined by measuring the pH value at half-neutralization with a Toadenpa HM 20B pH meter.

Kinetic Method.—The hydrolysis of ethyl dichloroacetate was carried out in an imidazole buffer (25 ml) at constant temperature by estimating the residual ester by a hydroxylamine assay.[5,19] The reaction was initiated by the addition of the ester (0.125 ml) in acetonitrile. At appropriate intervals aliquot portions (1.0 ml) were pipetted into a hydroxylamine mixture (0.50 ml) and left for 10 min at 30°. To this was added iron(III) chloride solution (1.0 ml) and the solution kept at 30° for 1 h. The absorbance of the solution was read at 540 nm on a Hitachi 124 spectrophotometer. Buffer free base concentrations were >0.03M, so that pseudo first-order reaction conditions were maintained. From the plot of ln (OD_t − OD_∞) *versus* time, the first-order rate was determined. Each run was at least duplicated. The individual rate constants (k_{obs}) are accurate to within ±8%. These data are given in Table 1 together with $k_{[Im]_t}$ and k_0. Second-order rate constants (k_{im}) shown in Table 2 were obtained from the slopes of the plots of the first-order rate constants against the buffer free base concentrations, and the buffer ratio was mostly 1.0. The pH of the solutions was measured before and after kinetic runs and found constant (±0.01 pH unit).

Analysis of Products.—The reaction of the ester with 2-(2-hydroxypropyl)imidazole under kinetic conditions was run for a while in a flask and then the mixture was evaporated to remove the unchanged ester at temperatures below 30°. The residue was dissolved in water and an ester function was examined by the hydroxylamine assay. A control experiment without the imidazole was also carried out.

Formation of an acylimidazole intermediate was sought by a u.v. repeat scanning technique on a Hitachi 200 spectrophotometer using 2-methylimidazole.

RESULTS

Plots of $(k_{obs} - k_0)/[Im]_t$ *versus* the fraction of free base concentration are shown for some 2-substituted imidazoles

TABLE 1

First-order rate constants, $k_{[Im]_t}$, and k_0 for the hydrolysis of ethyl dichloroacetate at 30° [a]

$[Base]_t$/ M	$[Base]_f$/ M	10^3k_{obs}/ min^{-1}	pH	$10^2k_{[Im]_t}$ [b]/ l mol^{-1} min^{-1}	10^3k_0/ min^{-1}
Imidazole					
0.10	0.049	11.5			
0.30	0.147	21.7	7.11	4.6	7
0.50	0.245	31.6			
2-Methylimidazole					
0.40	0.04	14.4			
0.70	0.07	18.0	7.85	1.8	7
1.10	0.11	27.8			
0.12	0.03	40			
0.60	0.15	76	8.06	7.0	32
1.08	0.27	109			
0.10	0.05	100			
0.50	0.25	141	8.10	11	87
0.90	0.45	189			
2-Ethylimidazole					
0.40	0.04	7.5			
0.70	0.07	11.4	6.75	1.3	3
1.10	0.11	16.3			
0.12	0.03	28.9			
0.60	0.15	45.7	7.52	38	24
1.08	0.27	65.3			
0.10	0.05	77			
0.50	0.25	103	7.94	54	70
0.90	0.45	119			
2-Isopropylimidazole					
0.40	0.04	6.8			
0.70	0.07	9.4	6.72	0.47	5.2
1.10	0.11	10.4			
0.12	0.03	18.8			
0.60	0.15	24.4	7.40	2.0	16
1.08	0.27	36.7			
0.10	0.05	50.5			
0.30	0.15	64.5	7.90	1.4	55
0.50	0.25	67.9			
0.70	0.35	58.6			
2-Hydroxymethylimidazole					
0.20	0.05	2.6			
0.60	0.15	7.2	6.61	1.3	0.7
1.00	0.25	13.4			
2-(2-Hydroxypropyl)imidazole					
0.10	0.025	7			
0.50	0.125	17	7.04	2.5	4.5
0.90	0.225	27			
0.10	0.05	27.2			
0.30	0.15	37.3	7.58	4.7	23
0.50	0.25	47.0			
0.90	0.45	65.3			
2-(1,1-Dimethyl-2-hydroxyethyl)imidazole					
0.10	0.05	27			
0.50	0.25	36	7.58	2.3	25
0.90	0.45	46			

[a] Acctonitrile (0.5% v/v) and ionic strength 1.0 (KCl). Ester concentration 3.0×10^{-3}M. [b] Second-order rate constant for total base concentration.

in Figure 1. Good linear plots with a zero intercept were observed in most cases,[20] but a downward curvature is seen for 2-isopropylimidazole. The second-order rate constants (k_{im}) for substituted imidazoles are summarized in Table 2 together with the pK_a values. The k_{im} values for 2-isopropyl- and 2-hydroxymethyl-imidazole were obtained from the data with a buffer ratio of 1 : 3. It was difficult to include the data for 2-t-butylimidazole because of its limited solubility. The curvature observed for 2-isopropylimidazole was attributed to association of the molecules. In order to confirm this, measurements of both conductivity

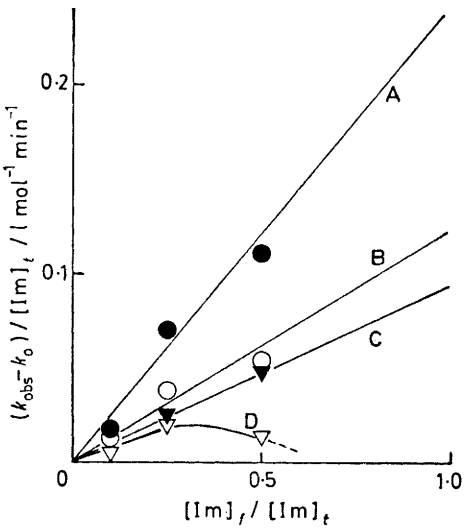

FIGURE 1 Plots of $(k_{obs} - k_0)/[Im]_t$ *versus* fraction of free base concentration of imidazoles: A, 2-methylimidazole; B, 2-ethylimidazole; C, 2-(2-hydroxypropyl)imidazole; D, 2-isopropylimidazole

in water and ^1H n.m.r. chemical shifts in deuterium oxide were carried out and the results are shown in Figure 2. A break is observed in these plots. The conductivity was measured at very low ionic strength, while the n.m.r. spectra were obtained under conditions similar to those of kinetic runs. Activation parameters were calculated from the data in Table 2, and are summarised in Table 3.

The possibility of acyl transfer to a hydroxy-group in the imidazole substituent was examined with 2-(2-hydroxypropyl)imidazole during hydrolysis. No ester formation was detected at roughly 70% conversion at 30°. The formation of an acylimidazole intermediate by attack of an

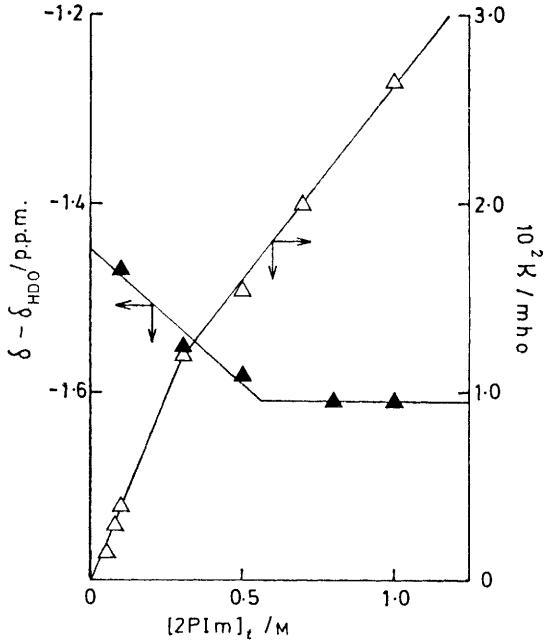

FIGURE 2 Plots of the conductivity (△) and ^1H n.m.r. chemical shift (▲) against the total concentration of 2-isopropylimidazole [2PIm]; δ for 1'-CH of 2PIm

TABLE 2

Substituted imidazole catalysed hydrolysis of ethyl dichloroacetate in water [a]

Imidazole substituent	pK_a [b]	10^2k_{im}/l mol^{-1} min^{-1} 20°	30°	40°	10^2R_X [c]/ l mol^{-1} min^{-1}	$-\log(R_X/R_H)$
1 2-H [d]	7.11	6.5	9.4		20	0
2 2-$HOCH_2$	6.73		5.7		18	0.034
3 2-Me	8.10	14	22	35	16	0.090
4 2-HOCH(Me)CH_2	7.53	5.8	9.3	15	13	0.20
5 2-Et	7.94		10		8.7	0.36
6 2-Pr^i [e]	7.90	3.9	8.0	9.8	7.2	0.44
7 2-$HOCH_2C(Me)_2$	7.58		4.6	9.3	5.9	0.53

[a] Reaction conditions are shown in Table 1. [b] At 30° and ionic strength 1.0. [c] Extrapolated rate value, see Figure 3. [d] At 10° 10^2k_{im} = 3.0 l mol^{-1} min^{-1}. [e] At 25° 10^2k_{im} = 6.0 l mol^{-1} min^{-1}. [f] At 48° 10^2k_{im} = 12 l mol^{-1} min^{-1}.

imidazole base upon the ester was checked by using 2-methylimidazole, the strongest base in the present series. No intermediate was observed in u.v. spectra.

TABLE 3

Activation parameters for substituted imidazole catalysed hydrolysis of ethyl dichloroacetate at 25° [a]

Imidazole substituent	$\Delta H^{\ddagger}$/ kcal mol^{-1}	$-\Delta S^{\ddagger}$/ cal mol^{-1} K^{-1}
2-H	9.1	41
2-Me	7.8	44
2-HOCH(Me)CH_2	8.7	45
2-Pr^i	7.5	47
2-$HOCH_2C(Me)_2$	9.6	41

[a] Calculated with second-order rate constants in l mol^{-1} s^{-1}.

DISCUSSION

The 2-position of the imidazole ring is a suitable site for substitution as discussed previously.[17] The behaviour of 2-isopropylimidazole at first appeared as if there was a general acid catalysis term. Although general base catalysis for hydrolysis of ethyl dichloroacetate has been shown to be governed by equation (1),[5]

$$k_{obs} = k_2[\text{base}]_{free} + k_0 \qquad (1)$$

it was not certain that the equation holds for substituted imidazoles. From experiments with different buffer ratios (Figure 1) it is concluded that equation (1) is also valid for the substituted imidazoles.[20] The anomalous behaviour of 2-isopropylimidazole is explained by a decrease in the effective concentration of the free base due to the formation of aggregates.[21,22] The data from the n.m.r. measurements seem to reflect a real situation in the reaction solutions, showing a break at a total base concentration of 0.6M. It is already known that intermolecular general acid catalysis is rarely observed in the hydrolysis of carboxylate esters.[2] Activation parameters (Table 3) show enthalpies of reasonable magnitudes and large, negative entropies, the latter being characteristic of a termolecular transition state where a molecule of water is involved in addition to the catalyst and substrate molecules. The values are comparable with those obtained for hydrolysis of cyclopentyl acetates [9] and *ON*-diacetylserine amide [23] catalysed by imidazole.

In the reaction of ethyl dichloroacetate with tris-(hydroxymethyl)aminomethane, Jencks and Carriuolo showed that the acyl group is transferred to the attacking base to form a stable product.[5] It was possible to expect that the hydroxy-group of the imidazoles participates covalently during hydrolysis. The absence of an ester function in the recovered buffer, 2-(2-hydropropyl)imidazole, may indicate either of the following: covalent participation of the hydroxy-group, followed by rapid hydrolysis of the intermediate (due to intramolecular imidazole catalysis) or the hydroxy-group did not participate at all. If the former is the case, the rate-determining step for hydrolysis must be initial nucleophilic attack by the oxygen atom. This is not compatible with the observed large negative entropy of activation. Therefore, the hydroxy-group does not participate. The possibility of direct nucleophilic attack by the imidazole nitrogen atom on the ester is also ruled out, since no intermediate acylimidazole is observed during hydrolysis with 2-methylimidazole, and because a large negative entropy of activation was observed with the same imidazole.[24]

The k_{im} values for the substituted imidazoles at 30° are not in the order expected from the size of the substituents (Table 2). This is partly due to the basicity difference among the imidazoles used. In order to evaluate the steric effect of the substituents, it is necessary to compare these rate constants with a standard. This may be done by assigning an extrapolated rate value (R_X) to each imidazole by making the following assumptions: each substituent series obeys the Brönsted catalysis law and there is an imidazole with a hypothetical pK_a of 7.80 in each substituent series. In the hydrolysis of ethyl dichloroacetate, Jencks and Carriuolo have shown a slope of 0.47 for a Brönsted plot for a series of relatively unhindred bases including imidazole.[5] Parallel plots of log k_{im} against pK_a with a common slope of 0.47 are shown in Figure 3. Although it is uncertain that different substituents obey a Brönsted plot with a single slope, the assumption may be allowed within a narrow range of pK_a. The extrapolated rate value (R_X) is obtained by taking the k_{im} value at pK_a 7.80 on the plot. A measure of the steric effect of a substituent in the imidazole is given by equation (2), where R_H represents the parent imidazole. Values of R_X and log (R_X/R_H) are also included in Table 2. Plots of log (R_X/R_H) *versus* the

$$\text{measure of steric effect} = \log (R_X/R_H) \qquad (2)$$

Taft steric constant parameters [25] are shown in Figure 4. Hydroxyalkyl groups are tentatively plotted on the same scale as the corresponding alkyl groups. Two

parallel lines with a slope of 0.26 are shown, an upper line representing the hydroxyalkyl groups and the bottom one the alkyl groups. The steric parameter sensitivity of 0.26 for these substituents is considerably smaller than that of 0.49 obtained for the substituents of the acyl portion of esters for a similar general base catalysed hydrolysis,[11] and makes a striking contrast with that of 1.33 for nucleophilic catalysis by the same imidazoles.[17] As shown in Figure 4, the hydroxy-group favours catalysis despite the larger steric demand than that for hydrogen. Bruice *et al.* explained the rate acceleration due to the presence of a vicinal hydroxy-group in the alcohol portion in terms of the inductive effect of the group.[9] There is no such complication in the present study. From the difference in the two parallel lines, a rate increase factor of 1.7 is calculated. It must be remembered that no allowance for the steric effect of the oxygen atom is made in this treatment. The rate enhancements [26,27] for ester hydrolysis by a neighbouring hydroxy-group have been considered in terms of hydrogen bonding,[28] acid–base catalysis,[29] nucleophilic reactions,[30] and solvent sorting or microscopic solvent effects.[27,31] The present case is explained most plausibly by this last effect. If the imidazoles participate solely as a proton base in the general catalysis, there would be no steric effect by the substituents. Both a small but sizeable steric effect and a hydroxy-group effect indicate that the substituent in the imidazole base is involved to some extent in the transition state of the catalysed reaction. It is known that there are a few hydrophilic functions along the periphery of enzyme active sites.[8] The role of the hydroxy-group in the imidazoles may be related to such functions. The small steric effect of the substituents may be compared with that found by Covitz and Westheimer in the pyridine bases.[32] The present

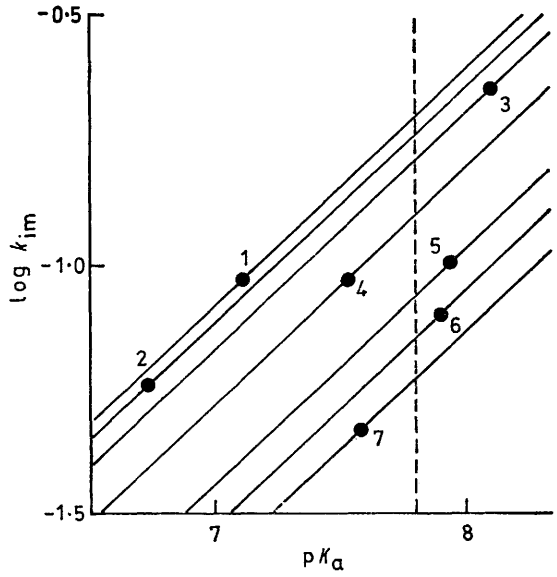

FIGURE 3 Parallel Brönsted plots to provide extrapolated rate values of substituted imidazoles with a hypothetical pK_a of 7.80. A common slope of 0.47 is assumed. For key, see Table 2

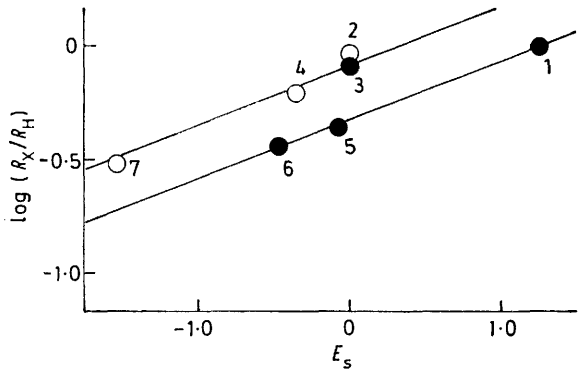

FIGURE 4 Plots of log (R_X/R_H) *versus* Taft E_s parameter: E_s values are given in ref. 25; hydroxyalkyl groups (○) are tentatively plotted on the same scale as the corresponding alkyl groups. For key, see Table 2

results show that the substituent effect of imidazoles upon general base catalysis is small.

We thank Mr. S. Ogawa and Mr. S. Miyahara for performing some experiments.

[9/002 *Received*, 2nd *January*, 1979]

REFERENCES

[1] T. C. Bruice and S. J. Benkovic, ' Bio-organic Mechanisms,' Benjamin, New York, 1966, vol. 1.
[2] S. L. Johnson, *Adv. Phys. Org. Chem.*, 1967, **5**, 237.
[3] W. P. Jencks, ' Catalysis in Chemistry and Enzymology,' McGraw-Hill, New York, 1969.
[4] M. L. Bender, ' Mechanisms of Homogeneous Catalysis from Protons to Proteins,' Wiley, New York, 1971.
[5] W. P. Jencks and J. Carriuolo, *J. Amer. Chem. Soc.*, 1961, **83**, 1743.
[6] B. M. Anderson, E. H. Cordes, and W. P. Jencks, *J. Biol. Chem.*, 1961, **236**, 455.
[7] J. F. Kirsch and W. P. Jencks, *J. Amer. Chem. Soc.*, 1964, **86**, 837.
[8] D. M. Blow, *Accounts Chem. Res.*, 1976, **9**, 145; R. Huber and W. Bode, *ibid.*, 1978, **11**, 114.
[9] T. C. Bruice, T. H. Fife, J. J. Bruno, and P. Benkovic, *J. Amer. Chem. Soc.*, 1962, **84**, 3012.
[10] M. Komiyama and M. L. Bender, *Bio-org. Chem.*, 1977, **6**, 13; J. B. Jones and K. E. Taylor, *Canad. J. Chem.*, 1977, **55**, 1653.
[11] J. B. Milstien and T. H. Fife, *J. Amer. Chem. Soc.*, 1968, **90**, 2164.
[12] G. M. Blackburn and H. L. Dodds, *J. Chem. Soc.* (*B*), 1971, 826.
[13] M. Utaka, J. Koyama, and A. Takeda, *J. Amer. Chem. Soc.*, 1976, **98**, 984.
[14] M. Komiyama, T. R. Roesel, M. L. Bender, M. Utaka, and A. Takeda, *Proc. Nat. Acad. Sci. U.S.A.*, 1977, **74**, 23; M. Komiyama, M. L. Bender, M. Utaka, and A. Takeda, *ibid.*, p. 2634.
[15] U. K. Pandit and T. C. Bruice, *J. Amer. Chem. Soc.*, 1960, **82**, 3386; J. A. Boudreau, C. R. Farrar, and A. Williams, *J.C.S. Perkin II*, 1978, 46.
[16] S. M. Felton and T. C. Bruice, *J. Amer. Chem. Soc.*, 1969, **91**, 6721; G. A. Rogers and T. C. Bruice, *ibid.*, 1974, **96**, 2473; T. H. Fife and D. M. McMahon, *J. Org. Chem.*, 1975, **35**, 3699; D. W. Griffiths and M. L. Bender, *Bio-org. Chem.*, 1975, **4**, 84.
[17] M. Akiyama, Y. Hara, and M. Tanabe, *J.C.S. Perkin II*, 1978, 288.
[18] A. Albert and E. P. Serjeant, ' The Determination of Ionization Constants,' Chapman and Hall, London, 1971, p. 65.
[19] S. Hestrin, *J. Biol. Chem.*, 1949, **180**, 249.
[20] W. P. Jencks, ref. 3, p. 163.
[21] J. H. Fendler and E. J. Fendler, ' Catalysis in Micellar and Macromolecular Systems,' Academic Press, New York, 1975, chs. 2 and 3.
[22] W. Lin, *Bull. Chem. Soc. Japan*, 1955, **28**, 227.

[23] M. J. Boland, M. J. Hardman, and I. D. Watson, *Bio-org. Chem.*, 1974, **3**, 213.
[24] M. L. Bender, ref. 4, p. 101.
[25] R. W. Taft, in 'Steric Effects in Organic Chemistry,' ed. M. S. Newman, Wiley, New York, 1956, p. 556.
[26] T. C. Bruice and S. J. Benkovic, ref. 1, p. 146.
[27] B. Capon and M. I. Page, *J. Chem. Soc.* (*B*), 1971, 741.
[28] S. M. Kupchan, J. H. Block, and A. C. Isenberg, *J. Amer. Chem. Soc.*, 1967, **89**, 1189.
[29] F. L. Killian and M. L. Bender, *Tetrahedron Letters*, 1969, 1255.
[30] B. Capon, S. T. McDowell, and W. V. Raftert, *J.C.S. Perkin II*, 1973, 1118.
[31] T. C. Bruice and T. H. Fife, *J. Amer. Chem. Soc.*, 1962, **82**, 1973.
[32] F. Covitz and F. H. Westheimer, *J. Amer. Chem. Soc.*, 1963, **85**, 1773.