Table I. Kinetic Parameters for Some α-Chymotrypsin-Catalyzed Reactions[a]

Substrate	Runs	$10^6[S]_0$, M	$10^6[E]_0$, M	$10k_0$, sec^{-1}	10^5K_0, M	k_0/K_0, M^{-1} sec^{-1}	Relative reactivity[b]
1	24	2.77–15.7	6.88	2.28 ± 0.14	1.05 ± 0.11	2.17×10^4	1×10^2
2	15	2.88–7.01	55.7	0.42 ± 0.00	1.05 ± 0.10	3.96×10^3	2×10
3[c]						$\sim 3 \times 10^3$	2×10
4	22	3.11–16.6	55.7	0.32 ± 0.02	1.00 ± 0.08	3.18×10^3	2×10
5	17	2.38–12.7	11.5	1.24 ± 0.07	0.35 ± 0.05	3.59×10^4	2×10^2
5[d]	28	2.38–12.7	55.4	0.042 ± 0.007	0.25 ± 0.01	1.65×10^3	
6	23	2.69–13.5	1.09	69.8 ± 3.1	0.41 ± 0.05	1.70×10^6	1×10^4
6[d]	24	2.69–13.5	11.1	1.61 ± 0.02	0.18 ± 0.01	9.0×10^4	
D-CDIC[e]				227	52.7	4.31×10^4	4×10^3[f]
L-CDIC[e]				1.24	11.7×10^2	1.06×10	1[f]
CIC[e]				1.34	141	9.50×10	9[f]
L-APNPE[g]				770	2.4	3.21×10^6	1×10^4[h]
NPA[i]						9.00×10^2	5

[a] Procedures were identical with those described in ref 5 and k_0 and K_0 are the usual steady-state parameters. Reactions were carried out at 25.3°, pH 7.95, in 20% methanol–3% acetonitrile unless otherwise indicated. [b] Based on k_0/K_0 under the conditions specified in *a*. [c] Estimated for a sample that contained considerable **4**. [d] At pH 5.4. [e] Reference 2, pH 7.9, 25°, water. [f] Estimated for the corresponding *p*-nitrophenyl ester assuming a100-fold increase for the change in leaving group and a 5-fold decrease for the solvent effect. [g] From B. Zerner, R. P. M. Bond, and M. L. Bender, *J. Am. Chem. Soc.*, **86**, 3674 (1964), pH 7, 25°, 3% acetonitrile. [h] Assuming a 2-fold increase for the pH correction and a 2.9-fold decrease for the solvent change. [i] Reference 5.

the light of (prejudices) (1–4) below. The four basic assumptions which furnished the ingredients for construction of Figure 2 are:

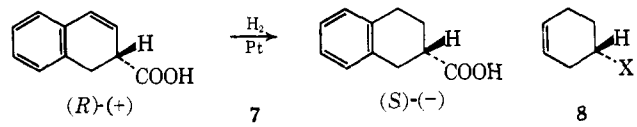
(1) The equatorial D-CDIC hypothesis is correct: that conformation of D-CDIC with an equatorial ester group (equatorial D-CDIC) is the one which undergoes hydrolysis by ChT and which should serve as the basic model for the reactive conformation of L-APME.[5]

(2) The amide groups of the reactive conformation of L-APME and of equatorial D-CDIC need not be oriented similarly. This assumption is supported both by the earlier argument that the amide group is not an important factor in determining the high reactivity of D-CDIC and by the observation that the amide group of D-CDIC is *cis* whereas that of L-APME is presumably *trans*.[5]

(3) The aromatic ring and ester group of the reactive conformation of L-APME should approximately coincide with the corresponding parts of equatorial D-CDIC.

(4) If the aromatic ring and ester group of any conformation of D-APME and the corresponding parts of equatorial D-CDIC are made to coincide, the acetamino side chains of D-APME and of the reactive conformation of L-APME should not have the same spatial orientation. This condition is introduced to reflect the stereospecific hydrolysis of L-APME by ChT.[11] Doubtless a similar procedure will yield a different model for the reactive conformation of L-APME if (1) is replaced by the axial D-CDIC hypothesis.[5]

If D-CDIC and **6** are truly analogous, **5** and **6** should have the absolute configurations indicated in Figure 1. Only tentative independent support for these assignments is now available. Dihydro acid of $[\alpha]^{25}D$ +93.5° (*c* 0.8, $CHCl_3$) was quantitatively hydrogenated (**7**) to tetrahydro acid with $[\alpha]^{25}D$ −18.1° (*c* 2, $CHCl_3$), which should have the (*S*) configuration if the carbon–carbon double bond of cyclohexene derivatives of type **8** and the benzo group of the tetrahydro acid may be equated.[12–14]



(*R*)-(+) **7** (*S*)-(−) **8**

Acknowledgments. All computations were performed at the University of Massachusetts Computer Center in Amherst. Mr. Stelios Arghyros assisted in the design of Figure 2, and Professor C. Peter Lillya measured many nmr spectra for us.

(11) If the phenyl and ester groups of D-APME are superimposed on the corresponding groups of the models shown in Figure 2, two extreme cases are observed. If the methylene groups of D- and L-APME coincide, the acetamino chain of D-APME lies in the direction of the hydrogen atom of the asymmetric carbon of L-APME, while if the methylene groups of D-APME and D-CDIC coincide, it lies in the direction of the CH→NH bond of D-CDIC.

(12) J. H. Brewster, *J. Am. Chem. Soc.*, **81**, 5493 (1959), has discussed the evidence that cyclohexene compounds of configuration **8** will be more levorotatory than their epimers.

(13) R. H. Pickard and J. Yates, *J. Chem. Soc.*, **89**, 1101 (1906), claim that completely resolved tetrahydro acid had $[\alpha]D$ −51.8° (*c* 1.4, $CHCl_3$). If this is true, the hydrogenation experiment leads to the prediction that completely resolved dihydro acid should have $[\alpha]D$ ±268°, in good agreement with our observed value for the (−) isomer.[6]

(14) The absolute configurations D. Battail-Robert and D. Gagnaire [*Bull. Soc. Chim. France*, 208 (1966)] have assigned to the 1,2,3,4-tetrahydro-2-naphthols are in accord with this argument.

(15) The Chemical Research Institute of Non-Aqueous Solutions, Tohoku University, Katahira-cho, Sendai, Japan.

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Polar and Solvent Effects on Homolytic Abstraction of Benzylic Hydrogen of Substituted Toluenes by *t*-Butoxy Radical

Sir:

In a number of cases polar substituent effects have been discerned in reactions in which a free radical attacks a molecule. For attack on benzylic hydrogen atoms, bromine[1] and chlorine[2] atoms and *t*-butoxy,[3,4] trichloromethyl,[5] and peroxy radicals[2] give rise to rate data best correlated by σ^+ parameters[6] except in the case of *t*-butoxy. Since the *t*-butoxy radical is elec-

(1) R. E. Pearson and J. C. Martin, *J. Am. Chem. Soc.*, **85**, 354 (1963).
(2) G. A. Russell and R. C. Williamson, Jr., *ibid.*, **86**, 2357 (1964).
(3) C. Walling and B. B. Jacknow, *ibid.*, **82**, 6113 (1960).
(4) R. D. Gilliom and B. F. Ward, Jr., *ibid.*, **87**, 3944 (1965).
(5) E. S. Huyser, *ibid.*, **82**, 394 (1960).
(6) The σ^+ correlations to free-radical reactions were introduced first by G. A. Russell, *J. Org. Chem.*, **23**, 1407 (1958).

Table I. Relative Reactivities of One Benzylic Hydrogen (k_a/k_d) of Substituted Toluenes toward the *t*-Butoxy Radical in Various Solvents at 45.0°[a]

Substituent	Solvents: Freon-113	Chlorobenzene	Acetonitrile
p-C_6H_5O	3.10	1.82	0.873
p-CH_3	2.88	1.73	0.663
m-CH_3	2.35	1.32	0.555
H	2.19	1.31	0.520
p-Cl	2.19	1.10	0.462
m-Cl	1.68	0.894	0.360
m-CN	1.37	0.871	0.311
p-CN	1.24	0.821	0.310

[a] k_a/k_d for one aromatic ring hydrogen was estimated as 0.14–0.16 in Freon-113 and 0.059 in chlorobenzene using $C_6H_5OC_6H_5$ or C_6H_5Cl as model substrates. Results in this table are corrected for the reactivities of ring hydrogens except for those in acetonitrile.

tron seeking in nature, it should display a pronounced polar effect in the transition state of hydrogen abstraction, and it seemed much likely to obtain a better correlation of rate data with σ^+ parameters than with σ.[2]

In general, the relative reactivities of the *t*-butoxy radical toward hydrogen donors are measured by the following competition

$$(CH_3)_3CO\cdot \xrightarrow{k_d} CH_3COCH_3 + CH_3\cdot \quad (1)$$

$$(CH_3)_3CO\cdot + RH \xrightarrow{k_a} (CH_3)_3COH + R\cdot \quad (2)$$

The ratio of the rate constant k_a/k_d can be calculated from the *t*-butyl alcohol/acetone ratio by the equation[7]

$$[(CH_3)_3COH]/[CH_3COCH_3] = (k_a/k_d)[RH]$$

By comparing the ratios of k_a/k_d for various compounds, the relative reactivities of RH toward the *t*-butoxy radical may be obtained; but recently Walling and Wagner have pointed out that the effects of solvents on these competitions are so significant that there are serious limitations on the determination of the relative reactivities using these techniques.[8] However, in a common solvent at high dilution, k_d must be kept constant no matter how significant the solvent effect may be, and then relative reactivities of RH in a solvent can be derived simply by comparing k_a/k_d values. Moreover, we expect to get other information on the solvent effect of radical reactions by comparing the series of k_a/k_d in different solvents.

We are now reporting the relative reactivities of benzylic hydrogen of substituted toluenes toward the *t*-butoxy radical using di-*t*-butyl peroxyoxalate[9] as a radical source in three different solvents: Freon-113, chlorobenzene, and acetonitrile.

Di-*t*-butyl peroxyoxalate was decomposed in excess (1:5) of substituted toluene of varying concentrations (0.05–0.2 *M*) in each of the solvents at 45.0°. Ratios of *t*-butyl alcohol/acetone were determined by gas chromatography. The plots of *t*-butyl alcohol/acetone *vs.* concentration of the substrates gave excellent straight lines, and the ratios of k_a/k_d were calculated by the method of least squares. The results are listed in Table I.

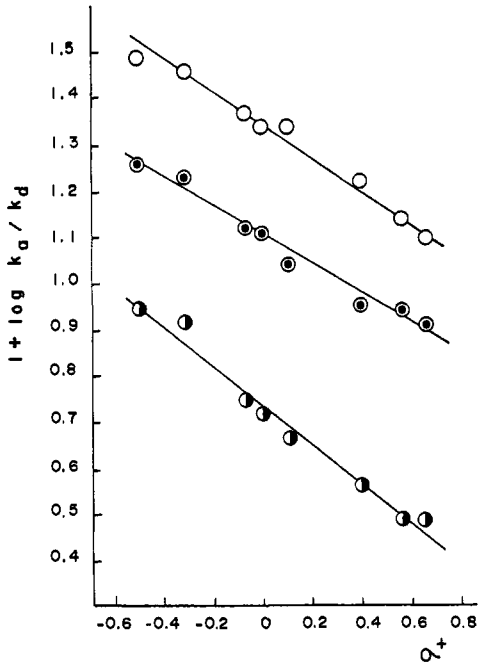

Figure 1. Correlation of $1 + \log(k_a/k_d)$ and σ^+ for abstraction of benzylic hydrogen of substituted toluenes by the *t*-butoxy radical in various solvents at 45.0°: -O-O-, in Freon-113; -◉-◉-, in chlorobenzene; -◑-◑-, in acetonitrile.

The data from Table I were fitted by the method of least squares to the Hammett equation with σ and σ^+.[10] The correlations are summarized in Table II.

Table II. Reaction Constants (ρ Values) and Correlation Factors

Solvent	With σ^+: ρ	r	With σ: ρ	r
Freon-113	−0.35	0.989	−0.40	0.977
Chlorobenzene	−0.32	0.973	−0.36	0.953
Acetonitrile	−0.39	0.994	−0.43	0.955

Figure 1 represents the relationship between log k_a/k_d and σ^+. Obviously the abstraction of benzylic hydrogen by *t*-butoxy radical follows a σ^+ rather than σ correlation, contrary to recent observation.[4]

Apparently, from Table II, only small effects of solvents on the reaction constant ρ are observed, while the k_a/k_d ratios vary widely.[11] However the variation of ρ from −0.32 to −0.39 with changes of solvent may represent some effect which suggests a dependence of k_a on solvent.[12]

(7) A. L. Williams, E. A. Oberright, and J. W. Brooks, *J. Am. Chem. Soc.*, 78, 1190 (1956).

(8) C. Walling and P. Wagner, *ibid.*, 86, 3368 (1964).

(9) P. D. Bartlett, E. P. Benzing, and R. E. Pincock, *ibid.*, 82, 1762 (1960). R. Hiatt and T. G. Traylor, *ibid.*, 87, 3766 (1965), demonstrated that cage recombination of *t*-butoxy radicals did actually occur in the decomposition of di-*t*-butyl peroxyoxalate in some extent depending on viscosities of the solvents, but these findings do not affect the present analysis.

(10) The σ and σ^+ constants are taken from C. D. Ritchie and W. F. Sager, *Progr. Phys. Org. Chem.* 2, 334 (1964).

(11) It would be possible that complexing of the *t*-butoxy radical with the added toluenes could also affect the rate of decomposition, k_d. Such effects of added olefin on the fate of higher *t*-alkoxy radicals, especially of benzyldimethylmethoxy radical, were observed by C. Walling and A. Padwa, *J. Am. Chem. Soc.*, 85, 1593 (1963). However, this sort of effect with a small amount of added toluenes may not be significant in the present study, because of excellent linear relations observed in each solvents.

(12) A referee pointed out that the variation in substrate toluene is only a minor structural change, so that the solvent effect reflected in σ would not be expected to be as large as the effects on k_d. Evaluation of ρ values for free-radical reactions in different solvents, however, seems to be an interesting problem.

It is interesting to note that our ρ values are quite different from those reported by Gilliom and Ward.[4] These authors used *t*-butyl hypochlorite as a *t*-butoxy radical source in competitive reaction conditions and reported ρ (with σ) at 40° to be −0.75. Interestingly there is a close resemblance between this value and the ρ reported for photochlorination of substituted toluenes (ρ = −0.76 with σ and −0.66 with σ^+ at 40°).[2]

We have no positive evidence to account for these discrepancies so far, but it is highly likely that ρ values derived from present data are directly related to the hydrogen abstraction reaction of *t*-butoxy radical. Identical ρ values for photochlorination of toluenes by chlorine[2] and by *t*-butyl hypochlorite[4] might suggest the involvement of the same propagating radical in both reactions, but there is strong evidence against this view.[13]

The discrepancies may be attributed to the differences in the mechanism (spontaneous decomposition *vs.* chain reaction), in the method (indirect *vs.* direct), or in other unknown factors. Recently Wagner and Walling[14] have pointed out that a complicated reaction took place in a competitive chlorination of the toluene–cyclohexane mixture with *t*-butyl hypochlorite by reasons which are still obscure. Related work is in progress.

Acknowledgment. We wish to thank Professors M. Kumada and O. Simamura for encouragement throughout the work and helpful discussions.

(13) C. Walling and A. Padwa, *J. Org. Chem.*, **27**, 2976 (1962).
(14) P. Wagner and C. Walling, *J. Am. Chem. Soc.*, **87**, 5179 (1965).

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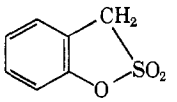
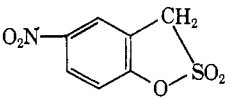
The Chymotrypsin-Catalyzed Hydrolysis of Sultones

Sir:

Recently we have been engaged in an investigation of the base-catalyzed hydrolyses of esters of sulfur-containing acids. We have found that certain five-membered cyclic sulfur-containing esters are extraordinarily labile to alkaline attack.[1,2] For example, the five-membered cyclic sulfonate, 2-hydroxy-α-toluenesulfonic acid sultone (I), undergoes hydroxide ion catalyzed hydrolysis 10^6 times faster than does the open-chain analog, phenyl α-toluenesulfonate. We now wish to report our observations on the unusual reactivity of the nitro-substituted sultone II[3] with a different type of nucleophilic species, the proteolytic enzyme α-chymotrypsin (CT), and the finding that II can be used to titrate the active sites of CT.



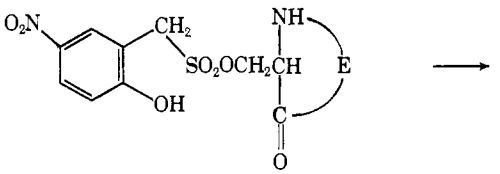
I



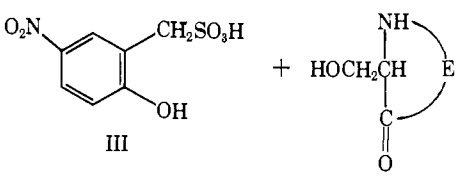
II

(1) E. T. Kaiser, I. R. Katz, and T. F. Wulfers, *J. Am. Chem. Soc.*, **87**, 3781 (1965).
(2) O. R. Zaborsky and E. T. Kaiser, *ibid.*, **88**, 3084 (1966).
(3) We thank Mr. K. W. Lo for a generous gift of II.

The hydrolysis of II to its product acid III (see below) was conveniently followed at pH values above 7 by spectrophotometrically monitoring the appearance of the phenolate ion peak near 400 mμ. CT does not absorb in this region. When II was added to a buffered solution containing CT (pH 7–8) a "burst" in the absorbance at 400 mμ, which was not observed in the absence of enzyme, occurred. The magnitude of this initial "burst" is determined by the species, enzyme or sultone, present in lower concentration. In the presence of excess enzyme, the "burst" was followed by a rise in absorbance to that of the product acid, III, at the given pH. These observations suggested that very rapid sulfonylation of the active site of CT by II to give the sulfonyl-enzyme IV occurred, followed by a slow decomposition of IV leading to the formation of the product III and regenerating the active enzyme.[4] We have performed several experiments which support this interpretation.



IV



III

chymotrypsin

The absorbance "burst" has λ_{max} 390 mμ, whereas λ_{max} for the product acid is 410 mμ. At 410 mμ and pH 7.6 the maximum absorbance "burst" is four-sevenths of the product acid absorbance. The different λ_{max} values and extinction coefficients indicate that we are dealing with a distinct intermediate species. If the "burst" represents a stoichiometric 1:1 reaction giving rise to a sulfonyl-enzyme, IV, it could provide a titration method for the determination of the concentration of CT active sites similar to that presently available using N-*trans*-cinnamoylimidazole (CI).[5] Titration with II at higher pH values is complicated by relatively rapid spontaneous hydrolysis of the excess sultone, making extrapolation of the "burst" to zero time nonlinear. But, as with CI, a "burst" is also produced at pH 5, this time at 320 mμ, the phenol absorption peak. Titrations at pH 5.05 of enzyme stock solutions with CI and with II gave essentially identical values for the active site concentration.[6]

(4) In structure IV we have assumed that the site of attachment of the sulfonyl group to the enzyme is a serine hydroxyl group. Evidence presented by many other investigators suggests that this is true for acyl-, phosphoryl-, and sulfonyl-chymotrypsins. Some recent pertinent reviews are: M. L. Bender and F. J. Kézdy, *Ann. Rev. Biochem.*, **34**, 49 (1965); T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," W. A. Benjamin, Inc., New York, N. Y., 1966, p 228–242.
(5) G. R. Schonbaum, B. Zerner, and M. L. Bender, *J. Biol. Chem.*, **236**, 2930 (1961).
(6) The extinction coefficient of the intermediate may be found by adding a known amount of sultone II to an *excess* of the enzyme at the desired pH. All of the sultone is then consumed, resulting in a "burst" at 320 mμ (and/or 390 mμ depending on the pH), which corresponds to the formation of a known amount of sulfonyl-enzyme and when extrapolated to zero time allows the calculation of the extinction coefficient of this species.