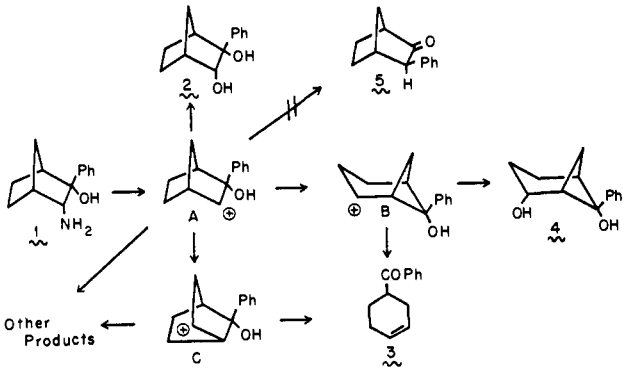
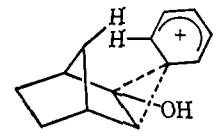


norbornylamine (**1**) the C_3–phenyl bond is *trans* (although about 60° away from coplanarity) with the C_2–N bond, a situation as conducive as possible for phenyl migration in the norbornane skeleton. Further, since migration of phenyl during deamination of **1** should lead to 3-*exo*-phenylnorbornanone-2 (**5**), one might suspect a powerful driving force for ketone formation. Accordingly we synthesized and deaminated **1** in order to determine whether—even under these favorable conditions—phenyl is still unable to undergo *exo–exo* shift to an adjacent carbon.

Deamination of the hydrochloride of **1**[10] in water led to all of the products previously[5] isolated during hydrolysis of 3-*exo*-*p*-toluenesulfonoxy-2-*endo*-phenylnorborneol-2, plus the three additional products **2**[11] (6%), **3**[11] (23%), and **4**[12] (22%). There was no evidence for the formation of 3-*exo*-phenylnorbornanone-2 (**5**).[13] Thus 7,2 Wagner–Meerwein rearrangement to form ion **B** and thence **4** is an easier pathway than *exo–exo* migration of phenyl.[14] Since the diol **4** is a major product of the deamination (22% yield) the ratio of 7,2 shift to phenyl migration (2,3-*exo–exo*) is at least 20:1 and may be much greater depending upon how much of the ketone **3** is produced through ion **B**. The reasons for this remarkable behavior do not appear to lie in the slightly unfavorable dihedral angle between the C_2–N and C_3–phenyl bonds, for this factor does not prevent the migration of hydrogen[3] or methyl[4] when similarly situated. We propose that in the transition state (**D**) for phenyl migration the σ-hydrogen of the



D

phenyl and the 7-*syn*-hydrogen of the norbornane skeleton interfere with each other to such an extent that phenyl migration is excluded.

(15) Atomic Energy Commission Postdoctoral Fellow, 1963–1965.

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The Pepsin-Catalyzed Hydrolysis of Sulfite Esters

Sir:

Beginning with the pioneering work of Fruton and Bergmann in 1939, a number of investigations have shown that the proteolytic enzyme pepsin catalyzes the hydrolysis of a limited class of N-acyl-L-dipeptides and N-acyl-L-tripeptides.[1-7] Recently it has been established that pepsin also catalyzes the hydrolysis of selected esters of β-phenyl-L-lactic acid.[8,9] We wish to report that certain organic sulfite esters are excellent substrates for pepsin.[10]

(1) J. S. Fruton and M. Bergmann, *J. Biol. Chem.*, **127**, 627 (1939).
(2) L. E. Baker, *ibid.*, **193**, 809 (1951); **211**, 701 (1954).
(3) M. S. Silver, J. L. Denburg, and J. J. Steffens, *J. Am. Chem. Soc.*, **87**, 886 (1965).
(4) A. J. Cornish-Bowden and J. R. Knowles, *Biochem. J.*, **96**, 71P (1965).
(5) W. T. Jackson, M. Schlamowitz, and A. Shaw, *Biochemistry*, **4**, 1537 (1965).
(6) E. Zeffren and E. T. Kaiser, *J. Am. Chem. Soc.*, **88**, 3129 (1966).
(7) K. Inouye, I. M. Voynick, G. R. Delpierre, and J. S. Fruton, *Biochemistry*, **5**, 2473 (1966).
(8) L. A. Lokshina, V. N. Orekhovich, and V. A. Sklyankina, *Nature*, **204**, 580 (1964).
(9) K. Inouye and J. S. Fruton, *J. Am. Chem. Soc.*, **89**, 187 (1967).
(10) Preliminary report presented at the Pacific Slope Biochemical Conference, Eugene, Ore., Aug 25–27, 1966. This research was supported in part by Grant GM 13446, U. S. Public Health Service. Kinetic constants for the enzymic hydrolysis of methyl phenyl sulfite were obtained by following the initial rate of production of phenol at 270 mμ using a Gilford 2000 spectrophotometer. The concentration of pepsin (twice recrystallized, Worthington Biochemical Corp.) was estimated from the absorbance at 278 mμ assuming a molar absorptivity of 50,900 l. mole^{-1} cm^{-1} determined by G. E. Perlmann (*J. Biol. Chem.*, **241**, 153 (1966)). The total enzyme concentration in these experiments was $[E_T] = 1.5 \times 10^{-5}$ *M*; initial substrate concentration was varied from $[S]_0 = 0.6$ to 3.8 m*M*. Buffers (0.10 *M* glycine hydrochloride) were adjusted to an ionic strength of 0.50 *M* with sodium chloride. The $\Delta\epsilon$ for complete hydrolysis of methyl phenyl sulfite is 1.14 ± 0.02 × 10^3 at pH 2.0 and pH 4.0.

Methyl phenyl sulfite was prepared by the method of P. Carré and D. Libermann, *Compt. Rend.*, **195**, 799 (1926); bp 65–66° (1.2 mm). *Anal.* Calcd for $C_7H_8O_3S$: C, 48.84; H, 4.68; S, 18.62. Found: C, 49.09; H, 4.77; S, 18.21.

Phenyl sulfite was synthesized by the method of A. Green, *J. Chem. Soc.*, 500 (1927); bp 130° (0.45 mm). *Anal.* Calcd for $C_{12}H_{10}O_3S$: C, 61.52; H, 4.30; S, 13.68. Found: C, 61.73; H, 4.34; S, 13.98.

p-Bromophenyl sulfite was prepared from *p*-bromophenol and thionyl chloride; mp 62.5–63.5°. *Anal.* Calcd for $C_{12}H_8Br_2O_3S$: C, 36.76; H, 2.06. Found: C, 37.01; H, 2.20.

N-Diazoacetyl-DL-norleucine methyl ester was prepared according to the method of Rajagopalan, *et al.*;[11] mp 52.5–56.5°. *Anal.* Calcd for $C_9H_{15}O_3N_3$: C, 50.79; H, 7.09. Found: C, 49.41; H, 6.94.

N-Carbobenzoxy-L-phenylalanyl-L-tyrosine was purchased from Cyclo Chemical Corp. and recrystallized from methanol–water. *Anal.* Calcd for $C_{26}H_{26}N_2O_6$: C, 67.52; H, 5.66. Found: C, 67.02; H, 5.99.

(11) T. G. Rajagopalan, W. H. Stein, and S. Moore, *J. Biol. Chem.*, **241**, 4295 (1966).

(10) The synthetic route employed was

$$\text{2,3-norbornanedione} \xrightarrow{\text{PhMgBr}} \text{3-keto-2-phenylnorborneol-2 oxime} \xrightarrow{\text{LiAlH}_4} \mathbf{1}$$

Elemental analyses and nmr and infrared spectra were all consistent with the assigned structure.

(11) The diol **2** was synthesized independently by lithium aluminum hydride reduction (in small yield) of 3-keto-2-*exo*-phenylnorborneol-2. Ketone **3** was prepared by the addition of phenylmagnesium bromide to 3-cyclohexenylcarboxaldehyde followed by oxidation with CrO_3 in pyridine.

(12) P. Yates and R. J. Crawford, *J. Am. Chem. Soc.*, **88**, 1561 (1966), obtained 2β-hydroxybicyclo[3.1.1]heptan-6-one through the acid-catalyzed rearrangement of 3-diazonorcamphor. A sample of this ketone, on treatment with phenylmagnesium bromide, was converted to the diol **4**, thus confirming its structure. We are indebted to Professor Yates, who kindly supplied us with a sample of his ketone.

(13) Compound **5**, which we had prepared previously,[3] could have been detected in yields of 1%.

(14) The α-terpineol isolated upon deamination of 2-*endo*-bornylamine [W. Hückel and F. Nerdel, *Ann.*, **528**, 57 (1937)] and α-fenchylamine (2-*endo*) [W. Hückel and U. Ströle, *ibid.*, **585**, 182 (1954)] can also be formulated as arising from 7,2 shift to give an intermediate similar to **B**.

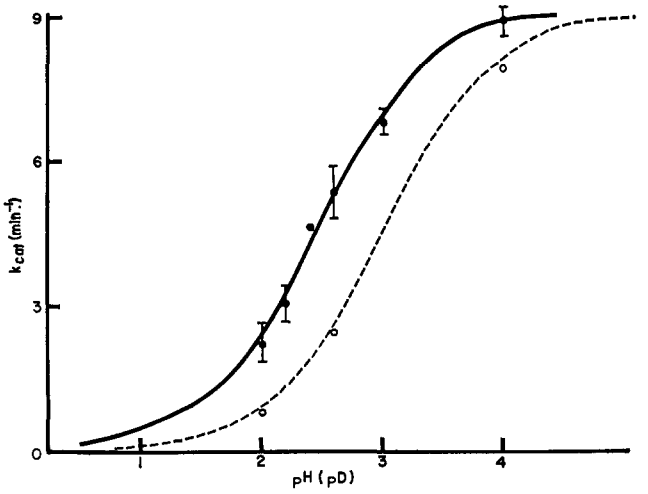

Figure 1. The catalytic constant, k_{cat}, for the pepsin-catalyzed hydrolysis of methyl phenyl sulfite *vs.* pH in water (—●—) and in deuterium oxide (—○—); 25.0°, μ = 0.5, 0.1 *M* glycine hydrochloride buffer. Curves are calculated from the equation $k_{cat} = k_{cat}^0/(1 + a_H/K_a)$ using $pK_a^{H_2O} = 2.6$ and $pK_a^{D_2O} = 3.1$.

We have studied the enzymic hydrolysis of methyl phenyl sulfite, phenyl sulfite, and *p*-bromophenyl sulfite. These compounds are readily synthesized and exhibit negligible rates of nonenzymic hydrolysis under our conditions; *e.g.*, the rate of the acid-catalyzed hydrolysis of phenyl sulfite is essentially 0 at pH 4 and 1×10^{-4} *M* ester ($k_{H_3O^+} = 0.16$ min^{-1} mole^{-1} l.).[12] Rates are conveniently determined by measuring the release of phenol spectrophotometrically and by measuring the release of protons in a pH-Stat; the same rate constants are obtained by either method. Table I gives kinetic data for three sulfite esters. The rate of ester hydrolysis is markedly influenced by the nature of the alcohol moiety; *p*-bromophenyl sulfite is 240 times more reactive than methyl phenyl sulfite.

Table I. Kinetic Constants for the Hydrolysis of Sulfite Esters Catalyzed by Pepsin[a]

Sulfite ester	k_{cat}/K_m, M^{-1} min^{-1} $\times 10^{-2}$	k_{cat}, min^{-1}	K_m, m*M*
Methyl phenyl	3.0	9.6 ± 0.6	32 ± 12
Phenyl	120 ± 6		
p-Bromophenyl	720 ± 32		

[a] 25.0°; 0.10 *M* glycine hydrochloride, pH 4.0, 0.2% (v/v) acetonitrile-water. Average deviations are reported. Three independent determinations of k_{cat} and K_m for methyl phenyl sulfite were used to obtain the reported average deviations; standard errors of about 20 and 45% were calculated for k_{cat} and K_m, respectively, by Wilkinson's method.[13]

The hydrolysis of methyl phenyl sulfite follows Michaelis–Menten kinetics; values for K_m and k_{cat} were calculated from a computer program of Wilkinson's weighted Michaelis–Menten equation.[13] The kinetics of the enzymic hydrolysis of phenyl and *p*-bromophenyl sulfite are first order in sulfite ester in the concentration range (2–10) $\times 10^{-5}$ *M*. The apparent lack of conformity to the Michaelis–Menten equation for these substrates may result from poor solubility which limits the concentration to 1×10^{-4} *M* in water. $[S]_0$ may not be able to approach K_m and the Michaelis–Menten equation $v_0 = k_{cat}[S]_0[E_T]/(K_m + [S]_0)$ approaches a second-order rate equation with $k_{obsd} = k_{cat}/K_m$. Fruton and co-workers[7] have reported the following data for N-carbobenzoxy-L-histidyl-L-phenylalanyl-L-tryptophan ethyl ester (pH 4.0, 37°): K_m = 0.23 m*M*, k_{cat} = 31 min^{-1}. Jackson, *et al.*,[5] have reported values of K_m = 0.84 m*M* and k_{cat} = 4.2 min^{-1} (pH 4.5, 37°) for N-acetyl-L-phenylalanyl-L-diiodotyrosine. By comparison, the sulfite esters are excellent substrates.

Competition studies with the substrate N-carbobenzoxy-L-phenylalanyl-L-tyrosine (Z-Phe-Tyr) indicate that the pepsin-catalyzed hydrolysis of sulfite occurs at the active site of pepsin. If the hydrolysis of phenyl sulfite and of Z-Phe-Tyr utilizes the same active site on the enzyme, Z-Phe-Tyr should competitively inhibit phenyl sulfite hydrolysis and the inhibitor constant, K_I, should be identical with the Michaelis constant.[14] For the determination of K_I, the inhibitor concentration was varied from 0.01 to 0.06 m*M* at each of three phenyl sulfite concentrations. Results plotted according to the method of Dixon[15] gave a value of K_I for Z-Phe-Tyr of 0.10 ± 0.02 m*M* (pH 2.0, 25°) from the intersection of three lines. This value is in satisfactory agreement with the value of 0.16 ± 0.08 m*M* (pH 2.0, 35°) for the K_m reported by Silver, *et al.*,[3] and suggests that the two substrates compete for the same active site.

This contention gains further support from studies in which pepsin was selectively inhibited by reaction with N-diazoacetyl-DL-norleucine methyl ester. Rajagopalan, Stein, and Moore[11] have shown that this reagent rapidly inactivates pepsin with the introduction of only 1 equiv of norleucine residue per mole of enzyme under selected experimental conditions. Pepsin which had been allowed to react with N-diazoacetyl-DL-norleucine methyl ester under their conditions and dialyzed against phosphate buffer (0.04 *M*, pH 2.0) has less than 1% of the original specific activity toward phenyl sulfite and methyl phenyl sulfite.

The pH–k_{cat} profile for the pepsin-catalyzed hydrolysis of methyl phenyl sulfite is presented in Figure 1. The unprotonated form of an ionizing group with a pK_a of 2.6 appears to be involved in the rate-limiting step; the pK_a value shifts to 3.1 in deuterium oxide. Recently Clement and Snyder[16] have presented a pH–k_{cat} profile for the pepsin-catalyzed hydrolysis of N-acetyl-L-phenylalanyl-L-tyrosine methyl ester in which the dissociated form of an ionizing group with a pK_a value of 1.6 appears to be implicated. Although pepsin contains a single O-phosphoserine residue, Perlmann[17] has shown that the phosphate group is not essential for enzymic activity. Thus the kinetically determined pK_a values probably can be assigned to a side-chain carboxyl group at the catalytic site.

Evidence that the reaction represents nucleophilic attack by a carboxylate anion and not the kinetically indistinguishable general base catalysis is given by the determination of a deuterium solvent kinetic isotope

(12) C. A. Bunton and G. Schwerin, *J. Org. Chem.*, **31**, 842 (1966).
(13) G. N. Wilkinson, *Biochem. J.*, **80**, 324 (1961).
(14) L. L. Ingraham, *J. Am. Chem. Soc.*, **79**, 666 (1957).
(15) M. Dixon, *Biochem. J.*, **55**, 170 (1953).
(16) G. E. Clement and S. L. Snyder, *J. Am. Chem. Soc.*, **88**, 5338 (1966).
(17) G. E. Perlmann, *ibid.*, **74**, 6308 (1952).

effect ($k_{cat}^{H_2O}/k_{cat}^{D_2O} = 1.1 \pm 0.1$ at pH 4.0) consistent with nucleophilic catalysis. This result also excludes general acid catalysis by the protonated imidazolyl group of the single histidine residue in pepsin. Since Clement and Snyder[16] have also reported the absence of a deuterium solvent kinetic isotope effect in the hydrolysis of a peptide substrate, it appears that the absence of general acid–base catalysis is the most distinctive feature of pepsin-catalyzed hydrolyses.

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Photochemistry of Aromatic Ions. Photolysis of Quaternary Anilinium Salts

Sir:

No direct evidence has been reported concerning the process preceding dissociation in the photolysis of substituted benzenes, although several derivatives have been studied in detail to show that phenyl radicals are produced.[1,2] In particular, ionic derivatives require some type of charge transfer in the production of these radicals.[1a,b] Three distinctly different paths leading to phenyl radicals are outlined for ArX^+Y^-. Path A

$$ArX^+ \xrightarrow{h\nu} [ArX^+]^* \longrightarrow Ar\cdot + X\cdot^+ \quad (A)$$

$$Y^- \longrightarrow [Y^-]^* \xrightarrow{h\nu} Y\cdot + e^-(solv)$$
$$e^-(solv) + ArX^+ \longrightarrow [ArX] \longrightarrow Ar\cdot + X\cdot \quad (B)$$

$$ArX^+Y^- \xrightarrow{h\nu} [ArX,Y]^* \longrightarrow Ar\cdot + X\cdot + Y\cdot \quad (C)$$

predicts no effect of changing anion and corresponds to the process generally cited for photolyses of nonionic materials.[1c] Path B has ample precedent in the literature concerned with generation and reactions of solvated electrons.[3] Path C involves direct charge transfer, requiring counterion proximity and suitable interaction.

Aryltrimethylammonium salts, p-$RC_6H_4N(CH_3)_3^+Y^-$ (I), have been studied. The positively charged nitrogen atom exerts only an inductive effect on the ring, yet such compounds are known to be readily reduced electrolytically[4] or by alkali metals.[5] Their photochemical behavior has not been reported.

Oxygen-free 1% methanolic solutions of I readily yield the corresponding hydrocarbon and trimethylamine upon irradiation.[6] Ethyl and isopropyl alcohols are also suitable, each yielding the corresponding aldehyde or ketone. Water and *t*-butyl alcohol are unsatisfactory. Formation of phenyl radicals is further confirmed by the complete absence of anisole among the products and observation of ethylene glycol at higher substrate concentrations.

Absorption of light by the aromatic ring appears to be necessary. Cyclohexyltrimethylammonium iodide was recovered unchanged after 17 hr. Irradiation of I (R = H, Y = C_6H_5O) in Pyrex for several days resulted in decomposition of the phenoxide ion, but no benzene was detected. In contrast, irradiation in quartz afforded a 51% yield after 6 hr. Removal of the nitrogen from direct attachment to the aromatic ring hinders the reaction; benzyltrimethylammonium iodide yields only 10% toluene after 14 hr.

A series of anions was investigated, and the results are shown in Table I. The percentage of recovered starting material was obtained in most cases by vapor phase chromatography; the salts decompose readily in the inlet to yield the corresponding dimethylaniline.[7] The uniformly high material balance indicates that the figures in the table are a valid although approximate reflection of the relative reactivities, which clearly vary markedly. Effective anions are those which are known to be easily photooxidized.[8] Path A must therefore be discarded.

Table I. Irradiation of p-$RC_6H_4N(CH_3)_3^+Y^-$ for 2 Hr in Methanol

R	Y	% ArH	% recovered[a]
H	I	61	28
H	$C_6H_5CO_2$	51	40
H	C_6H_5O[b]	50	
H	$C_6H_5CH_2CO_2$	14[c]	63
H	AcO	4	71
H	Cl	0	*d*
H	CHO_2	0	85
H	BF_4	0	80[e]
CH_3O	I	90	<1
CH_3	I	78	16
F	I	71	25
CH_3O_2C	I	0	
CN	I	0	>90[e]

[a] By vpc (see text). [b] Irradiation time 6 hr. [c] Plus 14% toluene. Yields were 55 and 69% after 14 hr. [d] Addition of 1 equiv of NaI resulted in a 60% yield of benzene after 4 hr further irradiation. [e] Isolated.

Results obtained with various *para*-substituted derivatives (Y = I) illustrate the fact that electron-donating substituents favor the reaction whereas the yield drops drastically with electron-withdrawing groups. This speaks strongly against path B, if one assumes that reactivity is proportional to electron-scavenging ability. Anbar and Hart found $\rho = +4.8$ for the reaction between aromatic molecules and hydrated electrons.[9] In addition, the efficacy of phenoxide ion in promoting the photolysis eliminates path B. Although phenoxide is an excellent hydrated electron source,[8a] such a process is not detectable in methanol, while phenoxy radical

(1) (a) W. E. Lee, J. G. Calvert, and E. W. Malmberg, *J. Am. Chem. Soc.*, **83**, 1928 (1961); (b) C. E. Griffin and M. L. Kaufman, *Tetrahedron Letters*, 773 (1965); (c) W. Wolf and N. Kharasch, *J. Org. Chem.*, 30, 2493 (1965); (d) N. Kharasch and A. I. A. Khodair, *Chem. Commun.*, 98 (1967).

(2) G. A. Razuvaev and Yu A. Ol'dekop, *Zh. Obshch. Khim.*, **19**, 736 (1949), and references cited therein.

(3) E. J. Hart, Symposium Chairman, "Solvated Electron," Advances in Chemistry Series, No. 50, American Chemical Society, Washington, D. C., 1965.

(4) (a) B. Emmert, *Chem. Ber.*, **42**, 1507 (1909); (b) V. Gutmann, G. Schöber, and K. Utvary, *Monatsh.*, **88**, 887 (1957); (c) L. Horner and A. Mentrup, *Ann. Chem.*, **646**, 49 (1961); (d) M. Finkelstein, R. C. Petersen, and S. D. Ross, *Electrochim. Acta*, 10, 465 (1965).

(5) A. J. Birch, *J. Proc. Roy. Soc., N. S. Wales*, **83**, 245 (1949).

(6) Irradiations were conducted using a bank of ten GE G25-T8 germicidal lamps with approximately 96% output at 2537 A. All analyses were performed by vapor phase chromatography, using Porapak Q solid substrate (Waters Associates, Inc.). Yields were calculated by comparison of peak area with a calibrated standard for each compound.

(7) A. D. Site, *J. Org. Chem.*, **31**, 3413 (1966).

(8) (a) G. Stein, ref 3, p 230; (b) L. I. Grossweiner and H.-I. Joschek, ref 3, p 279.

(9) M. Anbar and E. J. Hart, *J. Am. Chem. Soc.*, **86**, 5633 (1964).