with or without three-dimensional models.[7] It appears, however, that a general phenomenon is being observed, namely, a higher affinity of the L-amino acid derivatives I to nucleic acid helices, *e.g.*, rI–rC, rA–rU$_2$,[8] and calf thymus DNA.[9] Whether or not the more specific interaction exhibited by the L-amino acid derivatives *vs.* the D enantiomers I for nucleic acid helices is peculiar only to these types of salts is not yet determined. It is felt that a general phenomenon is being observed; *i.e.*, other L-amino acid derivatives, *e.g.*, peptides, etc., have a higher affinity than the corresponding D-amino acid derivatives for nucleic acid helices. Further work along these lines is in progress.

Acknowledgment. This work was supported by the Rutgers Research Council and by Grants GM-13597 and GM-15308 from the U. S. Public Health Service.

(7) It has been suggested by a referee that the difference may be due to a stereochemical effect of the R group in blocking the interaction of the amino group with the phosphate group of the nucleic acids (coil and/or helix).

(8) The stabilization of the triple-stranded helix rA–rU$_2$ by the L-proline derivative 7 is greater than by the D-proline derivative **8** (ΔT_M = 1.0 and 0.9° at 3.0 × 10^{-3} and 5 × 10^{-3} *M*); however there is no difference between the T_m of the rA–rU$_2$ in the presence of compounds **2** and **3**.

(9) The L-amino acid derivatives, *i.e.*, L-proline derivative **7** and L-lysine derivative **9**, stabilize the double-stranded helix of calf thymus DNA to a greater degree than the corresponding D enantiomers, compounds **8** and **10**, respectively.

(10) National Science Foundation Predoctoral Trainee, 1965–1968.

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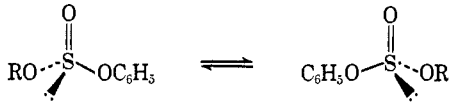
The Pepsin-Catalyzed Hydrolysis of Sulfite Esters. II. Resolution of Alkyl Phenyl Sulfites

Sir:

In recent years optically active enantiomers of sulfoxides,[1] sulfinate esters,[1] and sulfonium salts[2] have been reported, as well as rates of inversion for compounds containing a pyramidal sulfur atom.[3–5] Sulfite esters with two different groups should be resolvable into two enantiomers if the inversion barrier of the pyramidal sulfur atom is sufficiently high.



We wish to report resolution by the stereospecific pepsin-catalyzed hydrolysis of certain alkyl phenyl sulfites. This is the first report of the resolution of sulfite esters.[6] The finding that the hydrolysis of methyl phenyl sulfite is catalyzed by pepsin and that the salient features of this reaction are characteristic of enzymic reactions was reported in a previous communication in which the question of stereospecificity was not considered.[7]

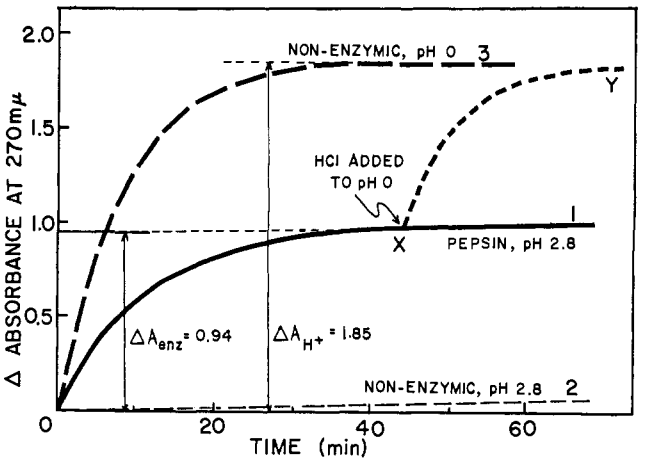


Figure 1. Time course of the enzymic and nonenzymic hydrolysis of phenyl tetrahydrofurfuryl sulfite (1.48 × 10^{-3} *M* in 0.1 *M* glycine and 0.5 *M* $LiClO_4$) at 25°: curve 1, in the presence of 8.2 × 10^{-6} *M* pepsin at pH 2.8; curve 2, in the absence of enzyme at pH 2.8; curve 3, in the absence of enzyme at pH 0; curve XY, addition of concentrated HCl at point X to lower pH of the reaction solution to pH 0.

Phenyl tetrahydrofurfuryl sulfite is an excellent substrate for pepsin.[8] Rates are measured by following the release of phenol spectrophotometrically at 270 mμ. Initial rates of hydrolysis conform to the Michaelis–Menten equation $v_0 = k_{cat}[S]_0[E_T]/(K_m + [S]_0)$.[9] Values of the steady-state kinetic parameters are k_{cat} = 3 min^{-1} and K_m = 1 × 10^{-3} *M* (25°; pH 2.8). However, the enzymic hydrolysis proceeds only to 50% completion (curve 1 of Figure 1), after correction for nonenzymic hydrolysis. The rate of the pepsin-catalyzed hydrolysis of the unreacted ester remaining after point X is not measurable, even at higher enzyme concentrations than that used in Figure 1. If the pH of the reaction solution is lowered to 0 by addition of concentrated HCl at point X, the remaining sulfite ester hydrolyzes (curve XY). Unreacted sulfite ester extracted at point X is optically

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(5) P. Turley and P. Haake, *J. Am. Chem. Soc.*, **89**, 4617 (1967).

(6) Examples of stereoisomerism arising from the pyramidal configuration of the sulfur atom in cyclic sulfite esters are known: (a) H. F. van Woerden, *Chem. Rev.*, **63**, 557 (1963); (b) P. C. Lauterbur, J. G. Pritchard, and R. L. Vollmer, *J. Chem. Soc.*, 5307 (1963); (c) E. J. Grubbs and D. J. Lee, *J. Org. Chem.*, **29**, 3105 (1964); (d) S. E. Forman, A. Durbetaki, M. Cohen, and R. Olofson, *ibid.*, **30**, 169 (1965); (e) H. van Woerden, D. van Valkenburg, and G. van Woerkom, *Rec. Trav. Chim. Pays-Bas*, **86**, 601 (1967).

(7) T. W. Reid and D. Fahrney, *J. Am. Chem. Soc.*, **89**, 3941 (1967).

(8) This research was supported in part by Grant GM 13446 and by a predoctoral traineeship (T. W. R.), Training Grant T1 GM-463, U. S. Public Health Service. Sulfite esters were prepared by the method of P. Carré and D. Liberman, *Compt. Rend.*, **195**, 799 (1926). *Anal.* Calcd for $C_{11}H_{14}O_4S$ (phenyl tetrahydrofurfuryl sulfite): C, 54.54; H, 5.83. Found: C, 54.95; H, 5.95. *Anal.* Calcd for $C_{12}H_{16}O_3S$ (phenylcyclohexyl sulfite): C, 59.99; H, 6.85. Found: C, 60.05; H, 6.71. These compounds were purified by low-temperature recrystallization from pentane. Their melting points were below −20°. Phenyl chlorosulfite (1.05 equiv) was allowed to react with racemic tetrahydrofurfuryl alcohol (1 equiv) in the presence of pyridine (1 equiv) at −20°. The product is a mixture of two pairs of enantiomers in equal proportions and showed no measurable optical rotation in a Cary 60 spectropolarimeter (240–450 mμ; 250 mg/ml, acetonitrile). The data presented in this communication clearly demonstrate that the presence of a second asymmetric center is not a necessary requirement for the stereospecific pepsin-catalyzed hydrolysis of this compound.

Kinetic constants were obtained by following the rate of production of phenol at 270 mμ with 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).

(9) $[E_T]$ is the total concentration of enzyme, $[S]_0$ is the concentration of the reactive enantiomer at zero time, and K_m is the Michaelis (apparent dissociation) constant of the enzyme–substrate complex.

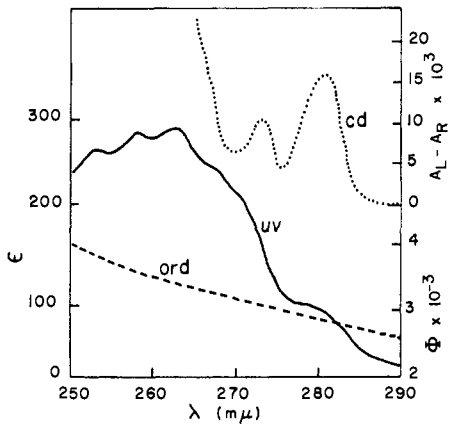

Figure 2. Ultraviolet, optical rotatory dispersion, and circular dichroism spectra of residual phenyl tetrahydrofurfuryl sulfite in acetonitrile. Optical path lengths: 1 cm. Concentration for CD spectrum: 4.25×10^{-3} *M*.

active.[10] Its infrared spectrum was identical with that of the original ester, which has no measurable optical rotation. The acid-catalyzed hydrolysis of the ester isolated after completion of the enzymic reaction can be followed spectropolarimetrically; the decrease in optical rotation follows first-order kinetics. The rotation was zero after 45 min at pH 0. These results provide convincing evidence that pepsin preferentially catalyzes the hydrolysis of one enantiomer of a sulfite ester.

The degree of stereospecificity exhibited in pepsin-catalyzed hydrolysis of alkyl phenyl sulfite esters is dependent on the structure of the alkyl group. The hydrolysis of methyl phenyl sulfite proceeds smoothly to 100% completion. The interpretation of this result is either that the small size of the methyl group may allow both enantiomers of this ester to form Michaelis complexes which undergo reaction at similar rates or that the less reactive enantiomer may racemize rapidly. The behavior of the phenyl cyclohexyl sulfite–pepsin system is intermediate, showing a distinctly biphasic reaction time course. Enzymic hydrolysis of this ester proceeds to 100% completion, one optical antipode reacting at least ten times faster than the other.

Figure 2 presents part of the circular dichroism spectrum of the unreacted phenyl tetrahydrofurfuryl sulfite ester isolated after enzymic hydrolysis.[11] The CD spectra from two independent experiments virtually superimpose. Interestingly the CD spectrum of recovered cyclohexyl ester is essentially identical; this suggests that the relative configuration of the more reactive enantiomer of each ester is probably the same. The structural requirements in the substrate which lead to stereospecific interactions with pepsin remain unanswered.

(10) For preparative runs conditions were the same as in Figure 1, curve 1. The reaction solution (250 ml) was extracted with 150 and 50 ml of dichloromethane after 45 min, corresponding to point X on curve 1. Combined dichloromethane extracts were washed with 5% aqueous sodium bicarbonate (three 30-ml portions), dried over magnesium sulfate–charcoal, and filtered. The solvent was removed under reduced pressure at 0°. The possibility that there was some contamination of the optically active sulfite ester by other optically active materials, *e.g.*, from degradation of the enzyme, is unlikely. First, control experiments showed that no extractable optically active material was formed in solutions containing enzyme only. Second, the acid-catalyzed hydrolysis of the extracted sulfite ester always resulted in complete loss of optical rotation.

(11) Circular dichroism studies were made on two Jasco-Durrum CD recording spectrophotometers made available to us through the kind cooperation of Dr. N. S. Simmons and Dr. W. F. H. Mommaerts. Both instruments show positive CD bands near 272 and 280 mμ, but the magnitude differs by 50% or less. The positive CD band(s) below 260 mμ are very large and have not been studied yet.

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Studies on Polypeptides. XXXVIII. Elimination of the Histidine Residue as an Essential Functional Unit for Biological Activity of β-Corticotropin[1-3]

Sir:

β-Corticotropin$_{1-20}$ amide (I),[4] a fragment of β-corticotropin[5] which in the rat possesses essentially the same *in vivo* adrenocorticotropic activity as the parent hormone,[6] was selected as a convenient molecule to investigate the effects of amino acid substitutions on biological activity.

In a previous study we synthesized 4-α-aminobutyric acid,5-glutamine-β-corticotropin$_{1-20}$ amide (II) and observed that this compound exhibited approximately 70% the biological activity of corticotropin A_1 on a weight basis.[7,8] From this result we concluded that the methionine sulfur is not essential for the biological function of ACTH. This conclusion was fully confirmed by Boissonnas, *et al.*,[9,10] who recorded the very high adrenocorticotropic potency of 625 IU/mg for 1-D-serine,4-norleucine,25-valine-β-corticotropin$_{1-25}$ amide.

We now wish to report the synthesis and some biological properties of 5-glutamine,6-β-(pyrazolyl-3)-alanine-β-corticotropin$_{1-20}$ amide (III).

The replacement of the histidine residue by β-(pyrazolyl-3)-alanine which results in a marked change in acid–base characteristics is not likely to alter significantly the conformation of peptide I since both imidazole and pyrazole are isosteric aromatic ring systems.[11]

In vivo corticotropic activity of homogeneous preparations of III was determined by three independent groups of investigators with essentially the same results.

The rat adrenal ascorbic acid depletion assay[12] showed compound III to possess approximately 50

(1) See F. M. Finn and K. Hofmann, *J. Am. Chem. Soc.*, **89**, 5298 (1967), for paper XXXVII in this series.

(2) The authors wish to express their appreciation to the U. S. Public Health Service for generous support of this investigation.

(3) Except for glycine the amino acid residues are of the L configuration. The following abbreviations are used: α-amino-*n*-butyric acid, Abut; β-(pyrazolyl-3)-alanine, Pyr(3)ala; DMF, dimethylformamide; DCC, dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; AP-M, aminopeptidase M (G. Pfleiderer, P. G. Celliers, M. Stanulovic, E. D. Wachsmuth, H. Determann, and G. Braunitzer, *Biochem. Z.*, **340**, 552 (1964)). Thin layer chromatograms were performed in the systems 1-butanol–acetic acid–water 60:20:20 (R_f^{I}) and 1-butanol–pyridine–acetic acid–water 30:20:6:24 (R_f^{III}).

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(8) H. B. F. Dixon and M. P. Stack-Dunne, *Biochem. J.*, **61**, 483 (1955).

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(11) K. Hofmann and H. Bohn, *J. Am. Chem. Soc.*, **88**, 5914 (1966).

(12) Ascorbic depleting activity was determined in 24-hr hypophysectomized rats according to the method of "U. S. Pharmacopeia," Vol. XV, Mack Publishing Co., Easton, Pa., 1955, p 176, against the USP reference standard.