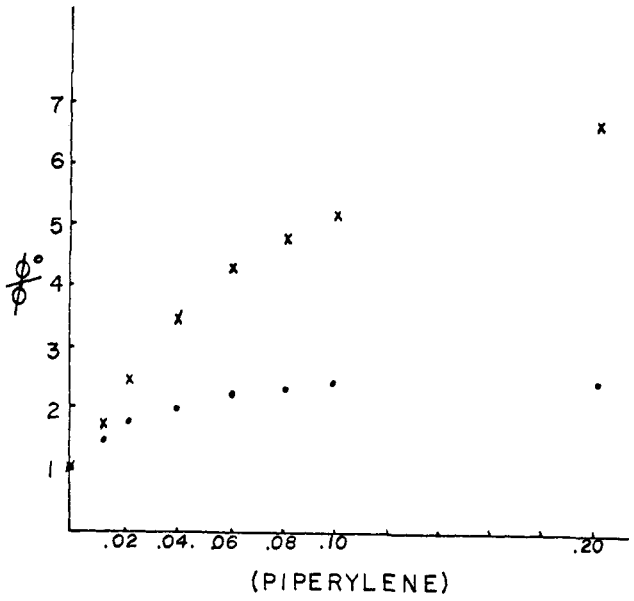


Figure 1. Quenching of oxetane formation by 1,3-pentadiene: ●, isomer **2**; X, isomer **3**.

indicate that *trans*-1-methoxy-1-butene (*t*-**1**) shows similar behavior.) *cis–trans* isomerization[10] of *c*-**1** to *t*-**1** competes with the cycloaddition reaction. Finally, the ratio (**2** + **3**):(**4** + **5**) is 1.4 for *both* singlet and triplet additions.

In the presence of 0.3 *M* **6** only singlet acetone (A_1) reacts with *c*-**1**, since **6** quenches acetone triplets (A_3) at close to the rate of diffusion[11] and the quenching of acetone triplets by *c*-**1** is at least 100 times less efficient (Figure 1). At low concentrations (*i.e.*, <0.1 *M*) of *c*-**1** in the absence of **6**, essentially only A_3 reacts with *c*-**1** because intersystem crossing is fast enough to compete with singlet cycloaddition. We may thus associate the initial slopes of Figure 1 with $k_q{}^3/k_1{}^3$, so that $k_1{}^3 = 2 \times 10^8$ l./mol sec.

$$A_3 + 6 \xrightarrow{k_q{}^3} A_0$$

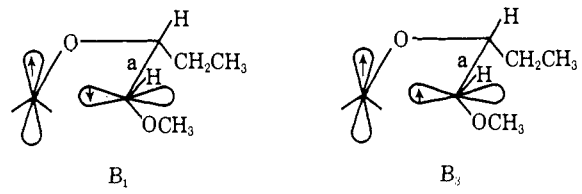
$$A_3 + c\text{-}1 \xrightarrow{k_1} \text{products and quenching}$$

In addition, a plot of $1/\Phi_2$ *vs.* $1/c$-**1** shows that $k_1{}^1/k_{st} = 0.53$, or $k_1{}^1 = 2 \times 10^8$ l./mol sec.

$$A_1 + c\text{-}1 \xrightarrow{k_1{}^1} \text{products and quenching}$$

$$A_1 \xrightarrow{k_{st}} A_3$$

The similarity of $k_1{}^1$ and $k_1{}^3$ and the increased stereospecificity associated with singlet addition imply a similar rate-determining attack by A_1 and A_3 on *c*-**1**, to form different intermediates, B_1 and B_3. Furthermore, although the stereospecificity which occurs at high [*c*-**1**] might suggest a precomplex of A_1 and *c*-**1**, *the fact that the same stereospecificity is observed at low c-1 when 6 is present* eliminates such a complex from consideration. Since the n orbital[3e] should direct an electrophilic attack of either A_1 or A_3 on the electron-rich C=C bond of *c*-**1**, we propose the shown structures for B_1 and B_3 immediately after their formation. B_3 cannot collapse to products without undergoing spin inversion; as a result, rotation around bond a is sufficiently fast relative to closure that essentially no



B_1 B_3

specificity is observed with respect to formation of **2** and **3**. B_1, on the other hand, may be an example of a "virtual biradical," *i.e.*, a biradical for which there may be sufficient bonding between the spin-paired but spatially separated electrons so that rotation is hindered or is slow relative to closure,[12] and, as a result, **2** is formed preferentially to **3**. Our results provide, for the first time, evidence of the comparative chemical behavior of a singlet and triplet 1,4 biradical which have similar and deducible geometry. Interestingly, these results imply that spin correlation between the electrons of 1,4 biradicals is sufficiently strong to affect the chemistry of these species.[13]

(10) The mechanism of this reaction is presently under investigation.
(11) P. J. Wagner, *J. Am. Chem. Soc.*, **89**, 2503 (1967); N. C. Yang and S. P. Elliott, *ibid.*, **90**, 4194 (1968).
(12) J. D. Roberts and C. M. Sharts, *Org. Reactions*, **12**, 9 (1962); see also E. F. Kieffer and M. Y. Okamura, *J. Am. Chem. Soc.*, **90**, 4187 (1968).
(13) (a) For related work on 1,4 biradicals see ref 7 and L. K. Montgomery, K. Schueller, and P. D. Bartlett, *J. Am. Chem. Soc.*, **86**, 622 (1964); (b) for recent studies directed toward the observation of differences which might result from radical pairs in solution which have their spins paired or unpaired, see J. R. Fox and G. S. Hammond, *ibid.*, **86**, 4031 (1964); S. F. Nelson and P. D. Bartlett, *ibid.*, **88**, 143 (1966); P. D. Bartlett and J. M. McBride, *Pure Appl. Chem.* **15**, 89 (1967); M. C. R. Symons, *Nature*, **213**, 1226 (1967).
(14) Alfred P. Sloan Fellow, 1966–1970.
(15) National Institutes of Health Trainee, 1965–1966.

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The Absolute Conformation of Chymotrypsin-Bound Substrates. Specific Recognition by the Enzyme of Biphenyl Asymmetry in a Constrained Substrate

Sir:

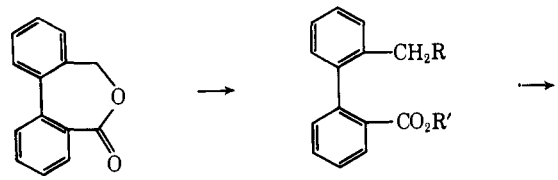
We submit an experimental solution to the problem of the conformation of α-chymotrypsin-bound (CT-bound) substrates possessing the natural L-phenylalanine pattern of structure.[1–7]

Our approach was facilitated by the discovery of an absolute specificity of CT toward a unique conformer of the 2,2′-bridged biphenyl analog (VI) of benzoylphenylalanine methyl ester ($BzPheOCH_3$), synthesized according to I → VI.

Treatment of diphenic anhydride with $NaBH_4$ in DMF[8,9] afforded the lactone I (87%), mp 136–137°.

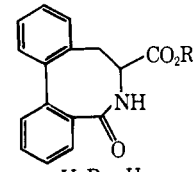
(1) B. F. Erlanger, *Proc. Natl. Acad. Sci. U. S.*, **58**, 703 (1967).
(2) G. E. Hein and C. Niemann, *ibid.*, **47**, 1341 (1961).
(3) E. S. Awad, H. Neurath, and B. S. Hartley, *J. Biol. Chem.*, **235**, PC35 (1960).
(4) S. G. Cohen and R. M. Schultz, *ibid.*, **57**, 243 (1967).
(5) I. B. Wilson and B. F. Erlanger, *J. Am. Chem. Soc.*, **82**, 6422 (1960).
(6) M. S. Silver and T. Sone, *ibid.*, **89**, 457 (1967).
(7) W. B. Lawson, *J. Biol. Chem.*, **242**, 3397 (1967).
(8) Abbreviations: DMF = dimethylformamide; EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline.[11]
(9) B. Belleau and J. Puranen, *Can. J. Chem.*, **43**, 2551 (1965).

Reaction of the latter[10] with PCl_5 in boiling CCl_4 followed by methanolysis gave II (82%), bp 133–135°



I

II, R = Cl; R′ = CH_3
III, R = $C(CN)(CO_2C_2H_5)NHCOCH_3$; R′ = CH_3
IV, R = $CH(CO_2^-)NH_3^+$; R′ = H

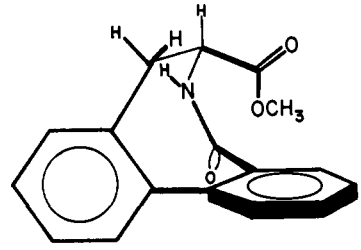


V, R = H
VI, R = CH_3

(0.1 mm). Condensation with ethyl acetamidocyanoacetate in ethanol–sodium ethoxide afforded III, which was hydrolyzed to the amino acid hydrochloride IV (62% based on II), mp 195° dec. Treatment of the latter with EEDQ[11] directly afforded V which was isolated as the methyl ester (diazomethane) VI (24%), mp 157–158°. The nmr spectrum (fresh $CHCl_3$ solution; relative to TMS) showed two singlets for methoxyl protons at τ 6.19 and 6.31 in a ratio of 70:30, as expected for two sluggishly interconvertible conformations of the tub-shaped eight-membered ring. The carbomethoxy can assume the equatorial (eq) orientation, thus projecting away from the phenyl rings, or can be axial (a), thus allowing for an anisotropic shift through interaction with the benzoyl moiety.

Incubation of racemic VI with CT in 95:5 water–dioxane at 25° (500 μg/ml; 10 μg of CT/ml) at pH 8 (continuous titration) caused hydrolysis to the extent of 25%. The resulting acid V gave a methyl ester, mp 135–137°, whose nmr spectrum showed one singlet at τ 6.31 (axial methoxyl protons; see above) provided a *fresh* $CHCl_3$ solution was used. A *fresh* dioxane solution gave rise to a strong Cotton band (ORD) at 252 mμ (35,000° amplitude), thus establishing that the biphenyl chromophore possesses the *R* configuration.[12] Contact with boiling HCl for 5 hr produced the amino acid V, which exhibited an ORD absorption spectrum characteristic of L-phenylalanine.[13] Therefore the enzymatically derived crystalline ester must possess the $R_{BiPH}S_{\alpha\text{-amido}}$ configuration with an *axially orientated* carbomethoxy (nmr data above). The molecule's absolute conformation is shown in formula VII (R,S_a conformer).

When a fresh solution of this conformer (in 95:5 water–dioxane) was incubated with CT *no hydrolysis* occurred. However, with an aged stock solution in dioxane CT-catalyzed hydrolysis took place readily. Hence, isomerization to an enzymatically active conformer was gradually taking place upon *destruction by*



R-S_a isomer
VII

dissolution in an organic solvent *of the crystal structure* of the R,S_a conformer. The kinetics of this isomerization could be followed by (a) the change in rotational strength at 252 mμ in dioxane, $CHCl_3$, and 95:5 water–dioxane, (b) the change in peak ratios in the nmr spectrum at τ 6.19 and 6.31, (c) rapid titration with a tenfold excess of CT (completion within 8 min). The relevant data are pooled in Figure 1, where agreement between the three methods is clearly established. Conformer VII's preequilibrium half-life in each solvent is given in Table I, which shows that the velocity of conformational change is drastically reduced in the aqueous solvent (95:5 water–dioxane).[15] It follows that isomerization of the R,S_a enantiomer VII to the S,S_{eq} substrate VIII occurs upon dissolution of the crystals. The apparent kinetic constants of this

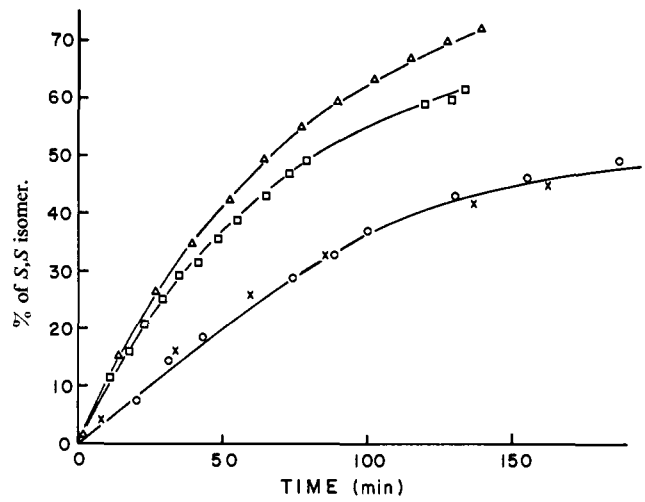
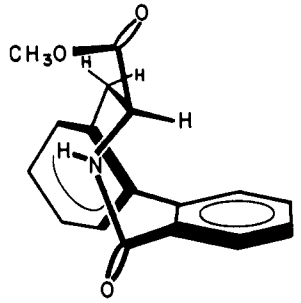


Figure 1. Time course of the conformational change VII → VIII: (×—×) by ORD in dioxane at 25° (rate of change of amplitude at 252 mμ); (○—○) by rapid titration with a 10 *M* excess of CT in 95:5 water–dioxane (at 25°) of dioxane aliquots of VII + VIII (at 25°); (△—△) by ORD in $CHCl_3$ at 28.5° (rate of change of amplitude at 252 mμ); (□—□) by nmr in $CHCl_3$ at 28.5° (rate of change of peak ratios at τ 6.19 and 6.31). In this latter case, intermolecular interactions decrease the equilibrium concentration of VIII by about 15% (compare with Table I). This complicating factor does not intervene at the low concentration used in the ORD measurements.

(10) All new compounds gave acceptable elementary analyses and had ir, nmr, and mass spectra consistent with the assigned structures.
(11) B. Belleau and G. Maleck, *J. Am. Chem. Soc.*, 90, 1651 (1968).
(12) E. L. Eliel, "Stereochemistry of Carbon Compunds," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 166.
(13) This assignment was confirmed by comparing the ORD curve of the N-nitroso derivative of the optically active ester VI with that of the N-nitroso derivative of L-pyroglutamic acid methyl ester, a model compound in which the amide function is also *cis*. The use of N-nitroso chromophores as derived from *trans* amides is well documented.[14]
(14) C. Djerrassi, E. Lund, E. Bunnenberg, and B. Sjöberg, *J. Am. Chem. Soc.*, 83, 2307 (1961).
(15) It is clear that hydrogen bonding between the amide function of VI and water strongly destabilizes the transition state for inversion (Table I). It is this quenching effect of water on the conformational change VII ⇌ VIII which allows determination of the time-dependent concentration of VIII with excess CT.



S-S_{eq} isomer

VIII

substrate as present in an equilibrated dioxane solution were: $K_m = 1.6 \times 10^{-3}$ and $V_{max} = 72$ μmol of CT/(min mg).[16] Atropisomerism and conformational

Table I. Relative Velocities of the Conformational Change VII → VIII[a] and Activation Energies[b]

Solvent	$t_{1/2}$ min,[c] VII → VIII	Equilibrium composition,[d] VII:VIII	$\Delta H^{\ddagger}$, kcal[e]
Dioxane	62	46:54	19 ± 0.5
Chloroform[f]	44[f]	<1:>99[f]	
Water–dioxane	488	64:36	29 ± 0.5

[a] In three solvents at 25° as measured by ORD at 252 mμ. [b] In two solvents. [c] Time required for 50% decrease in amplitude at 252 mμ relative to the equilibrium amplitude. [d] Obtained at $t_{1/2} \times 10$; after this time no detectable change at 252 mμ occurred. [e] From perfectly linear Arrhenius plots covering the temperature range 15–35° in the ORD cell. [f] At 30.2° instead of 25°.

asymmetry of a precisely definable nature in a substrate molecule are therefore recognized by CT. It is worthwhile emphasizing the fact that different conformations of VI are stabilized in the crystalline state (VII) and in solution (VIII), respectively. A careful comparison of molecular models leads to the conclusion that Hein and Niemann's substrate D-1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline and conformer VIII are related as a key is to its lock only when the ester function of the former is axially orientated in the CT-bound state, thus confirming earlier hypotheses,[2-5] while casting doubt on other interpretations,[1,6] including speculations on the absolute conformation of enzyme-bound $BzPheOCH_3$.[1] Other recent observations[19] with flexible substrates appear consistent with the conformational pattern set by the S,S_{eq} substrate VIII.

Acknowledgments. The authors are grateful to the National Research Council of Canada for the financial support of this work, to Dr. V. DiTullio for helpful suggestions, and to Dr. R. R. Fraser for assistance in the nmr measurements.

(16) For D-1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline[17] and L-$BzPheOCH_3$[18] the respective values are: $K_m = 5.3 \times 10^{-4}$ *M*, $V_{max} = 54.5$ μmol of CT/(min mg) and $K_m = 4.6 \times 10^{-3}$ *M*, $V_{max} = 90$ μmol of CT/(min mg).

(17) G. Hein, R. B. McGriff, and C. Nieman, *J. Am. Chem. Soc.*, **82**, 1830 (1960).

(18) J. E. Snoke and H. Neurath, *Arch. Biochem.*, **21**, 351 (1949).

(19) S. G. Cohen and A. Milovanović, *ibid.*, **90**, 3495 (1968).

(20) Holder of a National Research Council of Canada predoctoral scholarship, 1966–1968.

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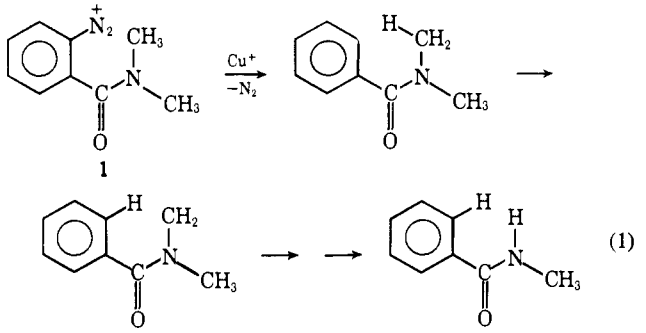
The Competition between Bond Rotations and Intramolecular Hydrogen Atom Transfer as Studied by the Use of Isotope Effects[1]

Sir:

The diazonium ion **1** derived from *o*-amino-N,N-dimethylbenzamide has the distinction of decomposing thermally to produce an aryl cation which undergoes a 1,5-*hydride ion* transfer while under the same conditions, but in the presence of copper compounds, the decomposition produces an aryl radical which undergoes a 1,5-*hydrogen atom* transfer.[2] The latter process which ultimately produces N-methylbenzamide is outlined in eq 1. We now wish to report a comparison of isotope



(1)

effects for these two types of hydrogen transfer and to illustrate a novel use of isotope effect data in the study of a rapid chemical reaction in competition with a conformational change.

Since these hydrogen transfers are rapid reactions occurring subsequent to the rate-determining steps, isotope effects must be determined by a competition method. When the diazonium ion **1**, labeled with three deuterium atoms in one of the methyl groups, is decomposed in water in the presence of cuprous oxide, the transfer of deuterium occurs to almost the same extent as that of hydrogen; the product isotope ratio, defined as the ratio of ring-undeuterated to ring-deuterated N-methylbenzamide, is 1.1. This would indeed be a unique case of a carbon–hydrogen bond cleavage if the virtual absence of isotopic discrimination were due to the lack of an isotope effect.[3] A far more likely explanation is that the rate of hydrogen atom transfer is much greater than that of rotation about the carbonyl C–N bond. It is this rotation which would equilibrate the two species (**2a** and **2b**) which are capable of undergoing the transfer. Since the two are not in equilibrium, the former would transfer only a hydrogen atom and the latter only a deuterium atom.[4] This bond rotation is known to be unusually slow in N,N-dimethylbenzamide itself, presumably due to the double bond character of this bond.[5]

This concept was tested by performing the radical decomposition of (1) the diazonium ion in which the

(1) This investigation was supported by Grant GP 7262 from the National Science Foundation.

(2) A. H. Lewin, A. H. Dinwoodie, and T. Cohen, *Tetrahedron*, **22**, 1527 (1966).

(3) K. Wiberg, *Chem. Rev.*, **55**, 713 (1955); L. Melander, "Isotope Effects on Reaction Rates," The Ronald Press, New York, N. Y., 1960, Chapters 4 and 6; K. Wiberg and E. L. Motell, *Tetrahedron*, **19**, 2009 (1963); A. F. Trotman-Dickenson, *Advan. Free Radical Chem.*, 1, (1965).

(4) The slight excess of hydrogen transferred can easily be accounted for by a slight degree of incomplete deuteration combined with an isotope effect. See below.

(5) M. T. Rogers and J. C. Woodbrey, *J. Phys. Chem.*, **66**, 540 (1962); L. W. Reeves, *Advan. Phys. Org. Chem.*, **3**, 252 (1965); R. C. Neuman, Jr., D. N. Roark, and V. Jonas, *J. Am. Chem. Soc.*, **89**, 3412 (1967).