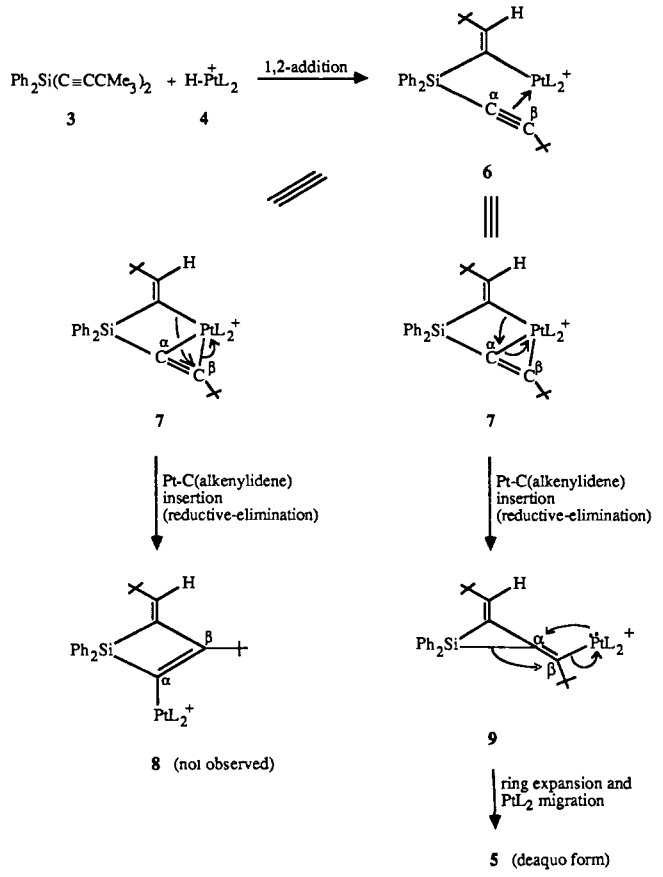
Scheme I



atomic numbering scheme is presented in Figure 1. The *trans*-$Pt(PEt_3)_2(H_2O)^+$ unit has the expected structural features and is bonded to the silacyclobutenyl ring through a Pt–C(3) alkenyl single bond of length 1.955 (9) Å.[17] The 4-alkylidene-1-silacyclobut-2-enyl ring system is characterized by an endocyclic C(2)–C(3) double bond distance of 1.40 (1) Å, an exocyclic C(4)–C(5) double bond distance of 1.35 (1) Å, and by a strained, intraring angle, C(2)–Si–C(4), of 75.3 (4)° centered at the Si atom. The corresponding distances and angle of the silacyclobutene ring in **2** are, respectively, 1.367 (11) Å, 1.334 (12) Å, and 74.0 (4)°.[8] Similarly, the values of the endocyclic C–C double bond distance and the internal angle centered at Si for compound **1** are 1.367 (4) Å and 78.6 (3)°, respectively. In complex **5**, both hydrogen atoms of the aquo ligand are involved in O–H···$FSbF_5^-$ hydrogen bonding. The O(W)···F(1) and O(W)···F(3′) [symmetry-related to F(3)] interatomic distances are 2.72 (1) Å and 2.75 (1) Å, respectively. These values fall within the normal range of 2.72 ± 0.09 Å for O···F interatomic distances in systems exhibiting O–H···F^- hydrogen bonding.[18]

A proposed mechanism for the formation of complex **5** is shown in Scheme I. Regioselective cis addition of the Pt–H bond of **4** across the C≡C bond of one of the alkynyl substituents of **3** would give a Pt, Si μ-alkenylidene intermediate, like **6**, in which the second alkynyl substituent of **3** coordinates to the cationic Pt(II) center. Reaction of **4** with the monoalkynyl phosphine oxide, $Ph_2(PhC{\equiv}C)P{=}O$, gives a complex of similar structure to **6** with the same regioselectivity for the Pt–H addition, $[Ph_2P(\mu\text{-}C{=}CPhH)(\mu\text{-}O)PtL_2]^+$.[19] Intermediate **6** can be represented formally as the oxidative-addition structure **7**. Direct insertion of the second alkynyl bond into the Pt–C(alkenylidene) bond of **7** can occur with two regioselectivities, as shown. Insertion whereby the Pt atom adds to C(α) of the alkynyl substituent would give the heterocyclic complex **8**. This compound is a structural isomer of **5**, and its formation is not observed. Insertion of the alkynyl substituent into the Pt–C(alkenylidene) bond whereby the Pt atom adds to C(β) of the alkynyl substituent would give a highly strained, silacyclopropane intermediate, such as **9**. Ring expansion and migration of the PtL_2 moiety, as shown, would give **5** prior to coordination of the water molecule. Other mechanisms which could effect the conversion of **3** and **4** to **5** have been proposed previously to rationalize the conversion of $O{=}P(C{\equiv}CCMe_3)_3$ and **4** to a similar Pt complex containing a 2-alkylidene-1,2-dihydro-3-phosphete *P*-oxide ligand.[19]

The general scope of this synthetic method and the chemical reactivity of compound **5** are under current investigation.

Acknowledgment. C.M.L. thanks the University Research Council of Vanderbilt University and the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. A platinum metal loan from Johnson Matthey, Inc., is gratefully acknowledged by C.M.L.

Supplementary Material Available: Tables of crystallographic data, atomic positional and thermal parameters, bond lengths and angles, torsion angles, and least-squares planes for complex **5** (14 pages); table of observed and calculated structure factors for complex **5** (34 pages). Ordering information is given on any current masthead page.

(16) Crystal data: $C_{36}H_{61}OP_2PtSiSbF_6$ (**5**), M = 1030.75, monoclinic, space group $P2_1/c(C_{2h}^5)$ - No. 14, a = 11.696 (4) Å, b = 17.341 (4) Å, c = 21.938 (9) Å, β = 91.40(2)° (from 25 orientation reflections, 33° < θ < 43°), V = 4448 (4) Å^3, Z = 4, d_{calcd} = 1.539 g cm^{-3}, μ(Cu Kα) = 121.8 cm^{-1}. Crystal dimensions: 0.11 × 0.15 × 0.20 mm. Intensity data ($+h$, $+k$, $\pm l$; 7942 nonequivalent, absorption-corrected reflections; θ_{max} = 67°) were recorded on an Enraf-Nonius CAD-4 diffractometer (Cu Kα radiation, λ = 1.5418 Å; graphite monochromator; ω–2θ scans). The crystal structure was solved by direct methods (MULTAN-11/82). Full-matrix least-squares refinement of atomic parameters (anisotropic C, O, P, Pt, Si, Sb, F; fixed H contributions) converged (max. shift 0.02σ) at R = 0.050 (R_w = 0.064, GOF = 1.52) over 4980 reflections with $I > 3.0\sigma(I)$. Further details are provided as Supplementary Material.

(17) Pt^{II}–C(alkenyl) distances normally fall within the range of 2.022 (8)–2.08 (2) Å. See: Hartley, F. R. *Comprehensive Organometallic Chemistry*; Wilkinson, G., Stone, F. G. A., Abel, E. W., Eds.; Pergamon Press: Oxford, 1982; Vol. 6, pp 471–762.

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Acyl Thrombin Photochemistry: Kinetics for Deacylation of Enzyme Cinnamate Geometric Isomers

Ned A. Porter* and John D. Bruhnke

Department of Chemistry, Duke University
Durham, North Carolina 27706

Received April 19, 1989

We recently reported[1] a means for photocontrol of the activity of the blood coagulation enzymes thrombin and Factor Xa. Compound **1a** was thus shown to inhibit thrombin and Factor Xa, and the inhibition was reversed by photolysis. There were, however, several problems with this inhibition–photoactivation strategy utilizing **1a**. The inhibition was temporary and enzyme activity returned after a few hours in the dark. Furthermore, the photoactivation of these enzymes was slow and required light intensities and wavelengths such that appreciable enzyme degradation occurred during photoactivation. Because of these problems, we have prepared several derivatives of **1a** and have studied the kinetics of the inhibition–photoactivation sequence in detail. We report here rate constants for our best inhibitor, **1b**,[2] and these

(1) Turner, A. D.; Pizzo, S. V.; Rozakis, G.; Porter, N. A. *J. Am. Chem. Soc.* **1988**, *110*, 244.

(2) The inhibitor **1b** was prepared by the procedures described for the preparation of **1a** in ref 1. All new compounds were fully characterized by spectroscopy and elemental analysis, and the details of the synthesis of **1b** and other analogues will be published in due course.

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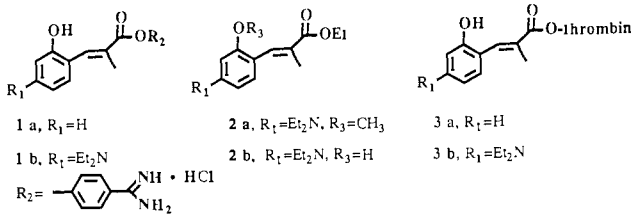
data allow a description of the time course of the photoactivation process.



The reaction of **1b** with thrombin was monitored by chromogenic assay.[1,3] A 1- to 5-fold excess of **1b** with thrombin (1.5 μM) in pH 7.4 Tris buffer led to complete loss of thrombin activity in less than 1 hour. Gel filtration of the resulting inactive thrombin solution on Sephadex G-25 with pH 7.4 Tris buffer solvent gave an inhibitor-free fraction eluting identically with active thrombin but with less than 2% activity. In the dark, thrombin activity of this solution increased in a clean first-order process with a rate of 1.4×10^{-6} s^{-1} (half-life for activation = 138 h). For comparison, the half-life for reactivation of **3a** was 3.8 h.

The *p*-diethylamino group of **1b** gives a characteristic red shift of the chromophore for **1–3** to 360 nm = λ_{max} compared to the parent (shoulder at 315 nm, λ_{max} = 280 nm). Assuming an ϵ of the acylenzyme **3b** equal to that of the corresponding ethyl ester **2b** (ϵ = 22 400), we conclude that the purified acylenzyme has one attached acyl group. These data support the notion that **1a** and **1b** acylate the serine active site hydroxyl of thrombin to give the acyl thrombins **3a** and **3b**. The *p*-diethylamino group of **3b** presumably donates electron density by resonance and stabilizes the acyl enzyme.[4]

We have studied the photochemistry of the model compounds **2a** and **2b** preliminary to our studies of the enzyme system. Photolysis of the methyl ether **2a** with 366-nm light results in a rapid decrease in absorbance as the cis photoisomer is formed. At the photostationary state,[5] the cis photoisomer is 60% of the mixture, and the ϵ of the cis compound is <40% of that of the trans at 360 nm. Photolysis for 5 min of the phenol **2b** in 98% ethanol/2% pH 7.4 Tris buffer gives a sharp decrease in the absorbance at 360 nm, followed by a slow increase in absorbance at 380 nm due to the dark formation of the coumarin **4**. The increase in the absorbance due to the coumarin is first order with $k_c = 7.17 \times 10^{-4}$ s^{-1}. The presence of the cis isomer has been confirmed by NMR after photolysis at <0 °C. The rate of cyclization of *cis*-**2b** is solvent dependent and increases by two orders of magnitude in 50/50 ethanol/Tris buffer (Table I). Photolysis of *trans*-**2b** in Tris buffer alone gives a clean conversion to coumarin with an isobestic point observed at 370 nm. Thus, in Tris and using conventional spectroscopy, there is no evidence for the formation of *cis*-**2b** in the conversion of the trans isomer to coumarin, but flash photolysis experiments (vide infra) indicate that the cis intermediate is formed but is very reactive in this solvent. The yield of coumarin from **2b** in essentially quantitative under all of the conditions described.

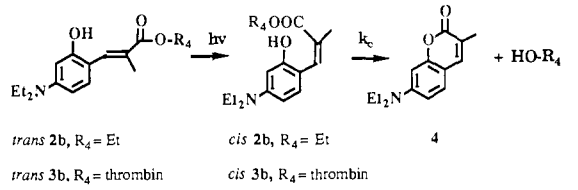
Photolysis of the acyl enzyme **3b** (2.0 μM in pH 7.4 Tris) with monochromatic 366-nm light for 25 s leads to the formation of fully active enzyme[6] (by chromogenic assay) and 1 equiv of coumarin **4**, as determined by gas chromatography and fluorescence of **4** at 480 nm. No evidence for a *cis*-acylenzyme photoisomer is seen by conventional spectroscopy. However, flash photolysis (10 ns) of the ethyl ester **2b** or the acyl enzyme **3b** in Tris buffer with 355-nm light from a Nd/Yag laser does give evidence for the cis photoisomer. For both **2b** and **3b**, the flash results in an immediate decrease in absorbance at 380 nm, followed by a first-order increase of absorbance as the coumarin forms from the cis intermediate. The important first-order rate constants determined in this study are presented in Table I.

Table I. First-Order Rate Constants for Enzyme Deacylation and Cyclization of Cis Photoisomers at 23 °C

compd	solvent	first-order rate constant, s^{-1}	half-life
trans-**3a**	Tris pH 7.4	$5.0 \pm 0.5 \times 10^{-5}$	3.8 h[a]
trans-**3b**	Tris pH 7.4	$1.4 \pm 0.2 \times 10^{-6}$	138 h[a]
cis-**2b**	98/2 ethanol/Tris pH 7.4	$7.2 \pm 0.2 \times 10^{-4}$	16.1 min[b]
cis-**2b**	50/50 ethanol/Tris pH 7.4	$9.7 \pm 1.4 \times 10^{-2}$	7.1 s[b]
cis-**2b**	2/98 ethanol/Tris pH 7.4	1.7 ± 0.5	0.4 s[b,c]
cis-**3b**	2/98 ethanol/Tris pH 7.4	$2.4 \pm 0.2 \times 10^{3}$	287 μs[b,c]

[a] Half-life of *trans*-acylenzyme deacylation. [b] Half-life of cyclization to give **4**. [c] Flash photolysis.

Scheme I. Photoisomerization–Thermal Lactonization to Coumarin 4



Comparison of the deacylation rates of the acylenzyme and the model ethyl ester is also of interest. Under the same conditions of solvent and temperature, *cis*-**3b** lactonizes 1000 times faster than the corresponding ethyl ester, *cis*-**2b**. The enzyme active site has a histidine–aspartic acid shuttle[9] to provide the requisite proton to the serine hydroxyl leaving group and to accept the proton from the phenolic nucleophile. The normal catalytic activity of the enzyme thus apparently assists in the deacylation once the internal nucleophile is presented to the active site by photoisomerization. Active site catalysis of processes such as dehydrohalogenation and lactamization of acyl serine proteases has been the subject of other important studies.[10]

The studies reported here suggest that this approach to photochemical control of enzyme activity may have diverse chemical and biological applications.[6,11] Critical to the success of such applications is the dynamics of the primary processes involved and the 10^9-fold rate difference in enzyme deacylation of the cis- and *trans*-enzyme photoisomers offers a dramatic kinetic response to photon absorption.

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(5) In all photolyses reported here, except for the laser flash studies, the source lamp was a mercury 500-W high pressure lamp. The 366-nm emission was isolated by a Bausch and Lomb grating monochromator.

(6) For alternate approaches for photochemical control of serine proteases, see: (a) Martinek, K.; Berezin, I. V. *Photochem. Photobiol.* **1979**, *29*, 637. (b) Berezin, I. V.; Kazanskaya, N. F.; Aisina, R. B.; Lukasheva, E. V. *Enzyme Microb. Technol.* **1980**, *2*, 150. (c) Kazanskaya, N. F.; Aisina, R. B.; Berezin, I. V. *Enzyme Microb. Technol.* **1983**, *5*, 209.

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(10) See, for example: (a) Kam, C.-M.; Fujikawa, K.; Powers, J. C. *Biochemistry* **1988**, *27*, 2547. (b) Krantz, A.; Spencer, R. W.; Tam, T. F.; Thomas, E.; Copp, L. J. *J. Med. Chem.* **1987**, *30*, 589. (c) Westkaemper, R. B.; Abeles, R. H. *Biochemistry* **1983**, *19*, 3256. (d) Hedstrom, L.; Moorman, A. R.; Dobbs, J.; Abeles, R. H. *Biochemistry* **1984**, *23*, 1753.

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Acknowledgment. This work was supported by a grant from PHS (HL-17921). We acknowledge generous gifts of thrombin from Professor S. Pizzo of Duke University. We also thank Dr. S. Atherton for help with the flash photolysis studies. These flash experiments and analyses of the data produced were performed at the Center for Fast Kinetics Research, which is supported jointly by the Biomedical Research Technology Program of the Division of Research Resources of NIH (RR00886) and by the University of Texas at Austin.

Cyclobutane as a General Ferromagnetic Coupling Unit. Design and Synthesis of a New, Hydrocarbon Quintet[1]

Julia A. Novak, Rakesh Jain, and Dennis A. Dougherty*

Contribution No. 7958, Arnold and Mabel Beckman Laboratories of Chemical Synthesis, 164-30 California Institute of Technology Pasadena, California 91125

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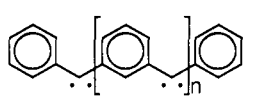
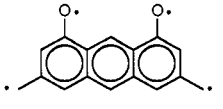
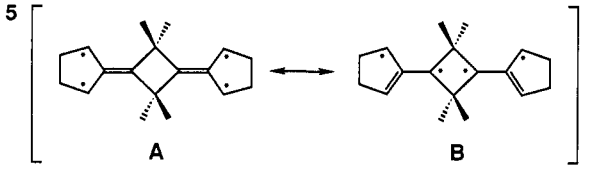
High spin states are commonly encountered among transition row metals and their complexes, arising from singly occupied d orbitals on the metal. High spin organic molecules, on the other hand, are a comparatively unnatural phenomenon, and their generation almost always results from a great deal of design and contrivance. In recent years, a series of organic molecules (**1**) with spin as high as $S = 6$ (multiplicity $= 2S + 1 = 13$; tridecet) has been documented, in which meta coupled phenyl carbenes provide the building blocks toward ever higher spin states.[2] These delocalized systems are based on a design which predicts high spin (ferromagnetic) coupling between adjacent units when the contiguous π topology of the molecule precludes the pairing of all electrons into bonds (non-Kekulé), provided the nonbonding MO's span common atoms (nondisjoint).[3] Similar π topology arguments have produced quintet tetraradical **2**.[4]



1

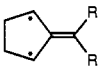


2



3

We present herein an example of an alternative ferromagnetic coupling mechanism between two triplets to give the higher spin state quintet ($S = 2$, four parallel spins). Tetraradical **3** combines two triplet 2-alkylidenecyclopentanediyls **4**,[5] derivatives of the classic non-Kekulé molecule, trimethylenemethane (TMM).[6] Coupling *between* the two TMMs occurs through the ground-state triplet, *localized*[7] biradical 1,3-cyclobutanediyl **5**,[8] as evidenced by resonance structure B.



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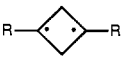
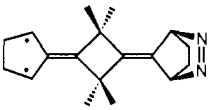


5

The synthesis (Scheme I) of the precursor bisdiazene **6**[9] required the bisfulvene, **7**.[9] This was prepared by capitalizing upon a creative application of Barton[10a] and Kellogg[10b] cycloaddition chemistry, first employed in related systems by Freund and Hünig.[11]

Photolysis[12] of **6** in either 2-methyltetrahydrofuran or poly(methylmethacrylate) matrices at temperatures between 4 and 77 K in the cavity of an EPR spectrometer results, after as little as 15 s irradiation, in the triplet spectrum shown in Figure 1a. This is a typical "powder" or randomly oriented triplet spectrum. The zero-field splitting (zfs) values, thermal stability, and hyperfine splitting in the $\Delta m_s = 2$ region (ca. 14 G) allow us to assign monoazobiradical **8** as the carrier of the signal. In particular, the spectral parameters closely match those of biradical **9** ($|D/hc| = 0.0255$ cm^{-1}; $|E/hc| = 0.0030$ cm^{-1}; hyperfine splitting = 13.5 G)[13] and a variety of other 2-alkylidene-1,3-cyclopentanediyls **4**.[5]



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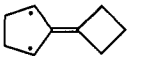

9

Further irradiation of the sample results in spectra typified by that shown in Figure 1b. In the $\Delta m_s = 1$ region, the six lines due to **8** are still evident. There are also many new lines which we assign to the quintet state of **3**, based on the following observations. The new lines, most clearly the two outer pairs (2600, 2710, 3830, and 3935 G), increase upon prolonged photolysis, while those due to **8** *decrease* in intensity. This suggests that the carrier of the new signal is photochemically derived from **8**, as expected for **3**. A quintet gives rise to a maximum of 12 transitions in the $\Delta m_s = 1$ region.[2,14,15] The two outer pairs completely determine the zfs values of a quintet (Figure 1) and allow prediction of the other transitions using a simulation program.[14a] As shown in Figure 1b, the quintet zfs values ($|D/hc| = 0.0207$ cm^{-1}; $|E/hc| = 0.0047$ cm^{-1}) produce good agreement between simulated and experimental spectra.[16] Interestingly, a new line is also evident in the $\Delta m_s = 2$ region of the spectrum (ca. 1700 G), a relatively unusual observation for $S = 2$ spectra.[14a]

We believe that the quintet is the ground state of **3** based on two observations. First, the spectra are quite intense at tem-

(1) Portions of this work were reported at the 196th National Meeting of the American Chemical Society, Los Angeles, CA, September 25-30, 1988.

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(6) Dowd, P. *Acc. Chem. Res.* **1972**, *5*, 242-248.

(7) The term "localized" is applied herein to biradicals in which the two radical centers are not in classical π conjugation; they may, however, be separately delocalized.[8]

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(9) **7**: ^{1}H NMR ($CDCl_3$) δ 1.69 (s, 12 H), 6.40 (m, 4 H), 6.50 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 30.1, 50.9, 120.2, 131.0, 137.4, 171.8. **10**: ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 12 H), 1.80 (m, 4 H), 2.00 (m, 4 H), 2.95 (s, 6 H), 4.67 (br s, 4 H). **6**: ^{1}H NMR (CD_2Cl_2) δ 1.03 (m, 4 H), 1.22 (s, 12 H), 1.60 (m, 4 H), 5.28 (br s, 4 H).

(10) (a) Barton, D. H. R.; Guziec, F. S.; Shahak, I. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1794-1799. (b) Buter, J.; Wassenaar, S.; Kellogg, R. M. *J. Org. Chem.* **1972**, *37*, 4045-4060.

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(12) EPR/photolysis conditions were as in ref 8.

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(14) (a) For a useful discussion of quintet EPR as it pertains to systems such as **3**, see: Seeger, D. E. Ph.D. Dissertation, Yale University, 1983. (b) Note that for larger D values, more than 12 lines can appear. See: Teki, Y.; Takui, T.; Itoh, K. *J. Chem. Phys.* **1988**, *88*, 6134-6145.

(15) Itoh, K. *Pure Appl. Chem.* **1978**, *50*, 1251-1259.

(16) A calculation of the D value for **3** along the lines of ref 2a and 2b produces $|D/hc| = 0.0212$ cm^{-1}. Details will be provided in the full account of this work.

0002-7863/89/1511-7618$01.50/0