23-2; [$PhCH_2$-Co$(DH)_2(PPh_3)$], 15977-36-3; [$PhCH_2$-Eco$(DH)_2$(P(c-$C_6H_{11})_3$)], 106095-15-2; [$PhCH_2$-Co(OEP)(PMe_2Ph)], 106095-16-3; [$PhCH_2$-Co(OEP)(P-n-Bu_3)], 106095-17-4; [$PhCH_2$-Co(OEP)-($PEtPh_2$)], 106095-18-5; [$PhCH_2$-Co(OEP)(PPh_3)], 106095-19-6; [$PhCH_2$-Co(OEP)(P(c-$C_6H_{11})_3$)], 106095-20-9; [$C_6H_5CH_2$Co(OEP)], 106095-21-0; PMe_2Ph, 672-66-2; P-n-Bu_3, 998-40-3; $PEtPh_2$, 607-01-2; PPh_3, 603-35-0; P(c-$C_6H_{11})_3$, 2622-14-2; vitamin B_{12}, 68-19-9; benzene, 71-43-2.

Evidence for Diazenyl Diradicals in the Photoisomerization of 4-Methylene-3,3,5,5-tetramethylpyrazoline

Waldemar Adam* and Markus Dörr†

Institute of Organic Chemistry
University of Würzburg, Am Hubland
D-8700 Würzburg, West Germany

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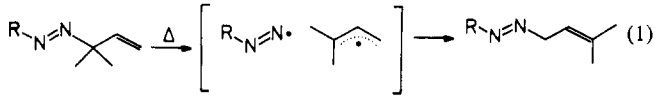
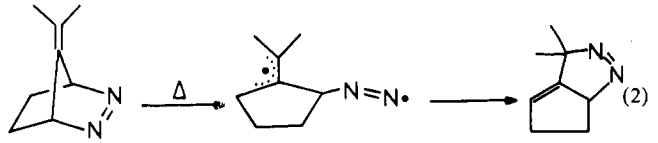
The mechanistic question whether extrusion of nitrogen from azo compounds proceeds concertedly or stepwise was recently[1] convincingly answered in the thermolysis of the unsymmetrically substituted acyclic azo compounds (eq 1). The fact that an



(1)

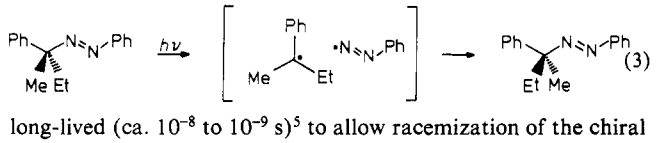
isomerized azo compound is formed, besides the usual denitrogenated products, implies rupture of the weaker C-N bond, leading to the caged diazenyl radical and allyl radical pair followed by recombination at the other allyl radical terminal prior to nitrogen loss from the diazenyl radical. Moreover, also for symmetrical bicyclic azo compounds, such thermal isomerizations have been documented[2] to proceed via diazenyl diradicals (eq 2). Theoretical



(2)

work substantiates[3] this mechanistic course, suggesting an appreciable activation energy (ca. 6-10 kcal/mol) for the loss of nitrogen from the diazenyl radical.

Experimental evidence indicates also for the photochemical process that on n,π^*-excitation photoracemization takes place (eq 3).[4] Again, the intermediary diazenyl radical is sufficiently

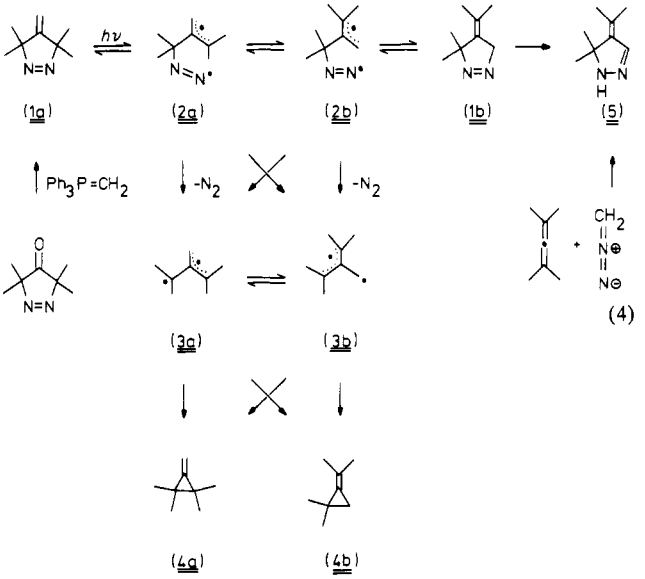


(3)

long-lived (ca. 10^{-8} to 10^{-9} s)[5] to allow racemization of the chiral alkyl radical, followed by cage recombination prior to nitrogen elimination. This is not surprising, since theoretical work shows,[6] with the parent diazene as model, that on n,π^*-excitation a π-type diazenyl radical is formed which should be reluctant toward denitrogenation. In this context, it is significant to mention that the bicyclic azo compounds in eq 2 did not isomerize during photolysis.[2]

In our recent publications,[7] we have proposed that diazenyl diradicals intervene quite generally in the photochemical denitrogenation of cyclic azo compounds. However, rigorous experimental proof is still lacking. Consequently, we decided to probe this mechanistic query by choosing an azo substrate capable of photoisomerization. Fortunately, the previously studied[8] 4-methylene-3,3,5,5-tetramethylpyrazoline (**1a**) proved useful for our purposes, and presently we communicate our results.

Azo compound **1a** was prepared in 75% yield from its pyrazolone by Wittig reaction with methylenetriphenylphosphorane.[8] Direct photolysis (ca. 0.7 M solution in *n*-pentane under a nitrogen atmosphere) at 350 nm and 20 °C afforded the methylenecyclopropanes **4a** and **4b** (eq 4) in the previously observed[8] relative



(4)

proportions of 75:25 (capillary GC on a 50-m OV-101 column, operated at column, injector, and detector temperatures of 80, 150, and 200 °C, respectively, and a carrier gas pressure of 1.0 kg/cm^2). However, careful examination of the photolyzate by means of capillary GC revealed a trace component (ca. 0.5%) of lower volatility, presumably still containing nitrogen.

This fact was indeed confirmed by means of capillary GC/MS, affording the expected *m/e* value of 138. Moreover, its fragmentation pattern was significantly different from the starting pyrazoline **1a**. That this product was not **1a** could readily be confirmed by means of capillary GC comparison with the authentic material.

Suspecting that the isomerized azo compound **1b** had been formed in the photolysis of **1a** (eq 4), we attempted to prepare an authentic sample. Cycloaddition of diazomethane and tetramethylallene in ether at 0 °C in an autoclave for 4 weeks gave

† Fonds der Chemischen Industrie doctoral fellow 1984-1986.

(1) Engel, P. S.; Gerth, D. B. *J. Am. Chem. Soc.* **1983**, *105*, 6849.
(2) (a) Cichra, D. A.; Duncan, C. D.; Berson, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 6527. (b) Berson, J. A. *Acc. Chem. Res.* **1978**, *11*, 446. (c) Cichra, D. A.; Platz, M. S.; Berson, J. A. *J. Am. Chem. Soc.* **1977**, *99*, 8507. (d) Berson, J. A. In *Diradicals*; Borden, W. T., Ed.; Wiley: New York, 1982.
(3) (a) Dannenberg, J. J.; Rocklin, D. *J. Org. Chem.* **1982**, *47*, 4529. (b) Hiberty, P. C.; Jean, Y. *J. Am. Chem. Soc.* **1979**, *101*, 2538.
(4) Porter, N. A.; Landis, M. E.; Marnett, L. J. *J. Am. Chem. Soc.* **1971**, *93*, 795.
(5) Bartlett, P. D.; Pincock, R. E.; Rolston, J. H.; Schindel, W. G.; Singer, L. A. *J. Am. Chem. Soc.* **1965**, *87*, 2590.
(6) Bigot, B.; Sevin, A.; Devaquet, A. *J. Am. Chem. Soc.* **1978**, *100*, 2639.
(7) (a) Adam, W.; Gillaspey, W. D.; Peters, E.-M.; Peters, K.; Rosenthal, R. J.; von Schnering, H. G. *J. Org. Chem.* **1985**, *50*, 580. (b) Adam, W.; Dörr, M.; Hill, K.; Peters, E.-M.; Peters, K.; von Schnering, H. G. *J. Org. Chem.* **1985**, *50*, 587. (c) Adam, W.; Oppenländer, T.; Zang, G. *J. Org. Chem.* **1985**, *50*, 3303. (d) Adam, W.; Hill, K. *J. Am. Chem. Soc.* **1985**, *107*, 3686.
(8) (a) Crawford, R. J.; Tokunaga, H. *Can. J. Chem.* **1974**, *52*, 4033. (b) Crawford, R. J.; Tokunaga, H.; Schrijver, L. M. H. C.; Godard, J. C. *Can. J. Chem.* **1978**, *56*, 998.
(9) Pale yellow wax, sublimated: mp 35-40 °C; IR (CCl_4) 3420, 3020, 3000, 2970, 1610, 1550, 1230, 1000, 980, 970 cm^{-1}; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.11 (s, 6 H, N-C(CH_3)$_2$), 1.26 (s, 1 H, NH), 2.12 (s, 3 H, =C-(CH_3)$_2$), 2.21 (s, 3 H, =C(CH_3)$_2$), 7.80 (s, 1 H, N=CH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.46 (q, =C(CH_3)$_2$), 20.07, (q, =C(CH_3)$_2$), 27.58 (q, N-C-(CH_3)$_2$), 51.55 (s, C-5), 123.10 (s, C=C), 131.75 (s, C=C), 167.49 (d, C-3); MS (70 eV), *m/e* 138 (18%, M$^+$), 95 (7%), 82 (100%), 67 (6%), 55 (6%), 54 (16%), 53 (7%), 41 (9%), 39 (17%). Anal. Calcd for $C_8H_{14}N_2$ (138.2): C, 69.52; H, 10.21; N, 20.28. Found: C, 69.51; H, 10.02; N, 20.46.

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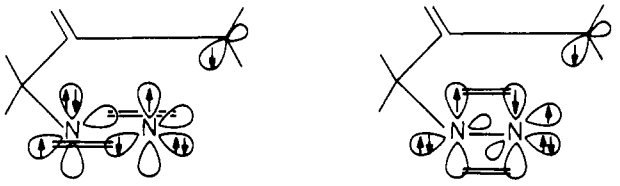
in trace amount (ca. 1%) the hydrazone **5** instead of the desired azo compound **1b**.[9] The minor product in the direct photolysis of azo compound **1a** was identical with hydrazone **5** by comparison with the authentic material by employing capillary GC/MS and GC/FTIR spectra and retention times on several capillary GC columns.

These results are mechanistically rationalized in eq 4. The most expedient way to explain the formation of hydrazone **5** is to accept the sequence **1a** ⇌ **2a** ⇌ **2b** ⇌ **1b** → **5**, involving first rupture of one C–N bond to afford the diazenyl diradical **1a**, which by C–C bond rotation leads to the rotameric diazenyl diradical **2b**, and final C–N bond reclosure yields the isomerized azo compound **1b**. The latter readily tautomerizes to the hydrazone **5**. Of course, the predominant competing reaction channel of the rotameric diazenyl diradicals **2a,b** is denitrogenation to afford the corresponding trimethylenemethane diradicals **3a,b**. Their subsequent fate is cyclization into the methylenecyclopropanes **4a,b**, respectively. The mechanistic intricacies of the latter process have recently been examined by means of deuterium labeling.[10]

What at first glance appears to be surprising about pyrazoline **1a** is the fact that its photolysis leads to isomerized azoalkane **1b** (eq 1), while its thermolysis does not,[8] although ample evidence for the involvement of diazenyl diradicals in the thermolysis of azo compounds has been documented.[11] A contrary case concerns the bicyclic azoalkanes of eq 2, in which the thermolysis led to isomerization but photolysis did not.[2] Even if structurally the "same" diradicals are involved in thermal and photochemical reactions, such species need not be identical. For example, they can differ in their vibrational excitation, i.e., "hot" vs. thermally equilibrated species,[12] in their spin states, i.e., singlet vs. triplet,[13] or in their electronic configuration,[14] i.e., σ- vs. π-type. Numerous examples exist which exemplify that the chemical fate of such molecular entities depends on such factors.[11] As we have postulated previously[7] on the basis of theoretical work,[6] photolysis (n,π^*-excitation) of azoalkanes should afford initially the π-type and thermolysis the σ-type diazenyl diradicals π-**2** and σ-**2**, respectively. While the latter is expected to readily denitrogenate



π - 2 σ - 2

to give the trimethylenemethane **3** and ground-state molecular nitrogen, the former would be obliged to generate **3** and n,π^*-excited molecular nitrogen. Thus, the photochemically produced diazenyl diradical π-**2** is expected to be longer lived, since nitrogen loss requires a greater activation energy and consequently formation of the isomerized azoalkane **1b** is more probable.[15]

It remains to be seen whether the intervention of diazenyl diradicals in the direct photolysis (n,π^*-excitation) of azo compounds is a general phenomenon, as seems to be implied in the theoretical study of the parent diazene.[6] It is our contention that diazenyl diradicals are more abundantly involved as intermediates than was previously implicated in the photolysis of cyclic azo compounds.

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Registry No. 1a, 55790-78-8; **1b**, 106251-08-5; **4a**, 54376-39-5; **4b**, 1121-36-4; **5**, 106251-09-6; diazomethane, 334-88-3; tetramethylallene, 1000-87-9.

(10) LeFevre, G. N.; Crawford, R. J. *J. Am. Chem. Soc.* **1986**, *108*, 1019.
(11) Dervan, P. B.; Dougherty, D. A. In *Diradicals*; Borden, W. T., Ed.; Wiley: New York, 1982.
(12) (a) Shen, K. K.; Bergman, R. G. *J. Am. Chem. Soc.* **1977**, *99*, 1655. (b) Stephenson, L. M.; Gibson, T. A. *J. Am. Chem. Soc.* **1974**, *96*, 5624.
(13) (a) Stephenson, L. M.; Brauman, J. I. *J. Am. Chem. Soc.* **1971**, *93*, 1988. (b) Bartlett, P. D.; Porter, N. A. *J. Am. Chem. Soc.* **1968**, *90*, 5317.
(14) Salem, L.; Rowland, C. *Angew. Chem.* **1972**, *84*, 86.
(15) Other cases involving π- vs. σ-type diradicals, see: (a) Adam W.; Berkessel, A.; Krimm, S. *J. Am. Chem. Soc.* **1986**, *108*, 4556. (b) Ito, Y.; Matsuura, T. *J. Am. Chem. Soc.* **1983**, *105*, 5237. (c) Adam, W. *Acc. Chem. Res.* **1979**, *12*, 390.

Nonenzymatic Sequence-Specific Cleavage of Single-Stranded DNA to Nucleotide Resolution. DNA Methyl Thioether Probes

Brent L. Iverson and Peter B. Dervan*

Arnold and Mabel Beckman Laboratories of Chemical Synthesis, California Institute of Technology Pasadena, California 91125

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We report the sequence-specific cleavage of single-stranded DNA to nucleotide resolution using chemically activated oligodeoxynucleotide methyl thioether hybridization probes (DNA-MT). 5-[3-[[3-(Methylthio)propionyl]amino]-*trans*-1-propenyl]deoxyuridine 5′-triphosphate (MT-dUTP) was enzymatically incorporated into an oligonucleotide duplex by using the Klenow fragment of DNA polymerase. Activation with CNBr at 25 °C, pH 5.5, followed by treatment with piperidine produced cleavage on the complementary strand at a single guanine (G) residue to the 5′-side of the modified base.

Oligonucleotide probes capable of producing cleavage at a complementary strand have been reported, based on modification of nucleic acid strands with bifunctional reagents.[1] Using (2-chloroethyl)amine derivatives attached to the 5′-terminal phosphate of an oligonucleotide, Vlassov et al. observed alkylation and cleavage of three adjacent G residues on a single-stranded DNA target.[2] A second synthetic approach has been developed involving incorporation of a modified base into an oligonucleotide by chemical methods which allows the convenience of automated synthesis and affords control over the placement of the reactive group in the oligonucleotide strand. One example of this is DNA–EDTA·Fe(II) probes which oxidatively cleave several nucleotides on the complementary strand.[4]

The work described here involves enzymatic incorporation of a modified deoxyuridine triphosphate into a specific site on an oligonucleotide. To avoid the problem of premature inactivation and/or autocleavage of the modified DNA probe and to control the timing of the complementary strand cleavage, the cleaving function is masked. On the basis of the known sequence-specific cleavage of peptides with cyanogen bromide (CNBr) which results from the selective conversion of methionine to a highly reactive sulfonium species,[6] methyl thioether covalently attached to a DNA hybridization probe could be considered a latent alkylating moiety that can be selectively activated by CNBr under mild conditions.

(1) For a review on "complementary addressed modification of DNA", see: Knorre, G. D.; Vlassov, V. V. *Prog. Nucl. Acid Res. Mol. Biol.* **1985**, *32*, 291 and references cited there.
(2) (a) Vlassov, V. V.; Gaidamakov, S. A.; Gorn, V. V.; Grachev, S. A. *FEBS Lett.* **1985** *182*, 415. (b) Vlassov, V. V.; Zarytova, V. F.; Kutiaven, I. V.; Mamaev, S. V.; Podyminogin, M. A. *Nucl. Acids Res.* **1986** *14*, 4065. See also ref 3.
(3) (a) Grineva, N. I.; Karpova, G. G. *FEBS Lett.* **1973**, *32*, 351. (b) Grineva, N. I.; Karpova, G. G.; Kuznetsova, L. M.; Venkstern, T. V.; Bayer, A. A. *Nucl. Acids Res.* **1977**, *4*, 1609. (c) Summerton, J.; Bartlett, P. A. *J. Mol. Biol.* **1978**, *122*, 145.
(4) Dreyer, G. B.; Dervan, P. B. *Proc. Natl. Acad. Sci.* **1985**, *82*, 968. For other synthetic approaches to DNA–EDTA probes, see ref 5.
(5) (a) Chu, C. F.; Orgel, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 963. (b) Vlassov, V. V.; Gaidamakov, S. A.; Gorn V. V.; Grachev, S. A. *FEBS. Lett.* **1985**, *182*, 415.
(6) Gross, E.; Witkop, B. *J. Am. Chem. Soc.* **1961**, *83*, 1510.

0002-7863/87/1509-1241$01.50/0