Stereochemistry of a [2 + 2] Cycloaddition of Cyclopentyne

J. C. Gilbert* and M. E. Baze

Department of Chemistry
The University of Texas at Austin
Austin, Texas 78712

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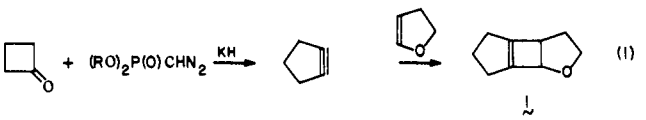
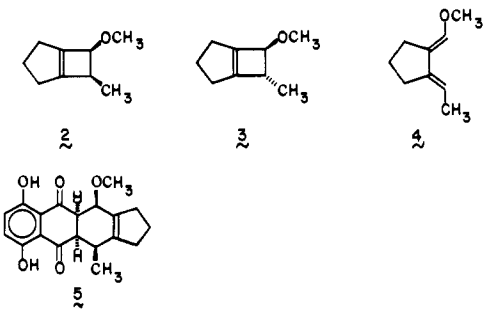
It was recently reported that base-promoted reaction of cyclobutanone with dialkyl (diazomethyl)phosphonates (DAMP) afforded cyclopentyne (eq 1).[1,2] One part of that study involved



(1)

production of a [2 + 2] cycloaddition product **1** between the alkyne and dihydrofuran, a reaction first reported by Fitjer et al.[4] The present report defines the stereochemistry of this type of cycloaddition.

Reaction of cyclobutanone, the anion of DAMP (generated with KH at −78 °C), and *cis*-1-methoxy-1-propene[5,6] afforded a cycloadduct which was isolated by HPLC and characterized by standard spectroscopic methods as being **2**.[7] Most relevant to



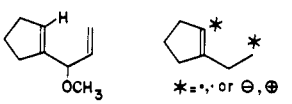
2 **3** **4**

5

the structural assignment are the similarity of the ^{13}C NMR spectra of **2** and **1** and the existence of a 3–3.5-Hz cis coupling constant between the protons α to the methoxy and methyl groups; this is the magnitude expected in such cyclobutenes, the trans coupling constant typically being no greater than 1.8 Hz.[8]

Repetition of the reaction with the *trans*-alkene gave a mixture of products in which the anticipated cycladduct **3** could not be detected spectroscopically even in the crude reaction mixture. However, the presence of olefinic resonances in the ^1H NMR spectrum raised the possibility that a diene, presumed on mechanistic grounds to be **4**, had been formed. This suspected diene was itself too labile to permit isolation by chromatographic methods but could be characterized by conversion to a Diels–Alder adduct having the structure **5** as shown by X-ray crystallographic analysis. The adduct has the cis methyl–methoxy relationship required if **4** had been generated by electrocyclic ring opening of the cycloadduct **3**.[9,10]

As revealed by ^1H NMR analysis the yields obtained of **2** and **4** were 22% and 20%, respectively, based on unrecovered cyclobutanone. Analysis by temperature-programmed GC-MS allowed definition of the stereochemistry of the reaction. The volatiles arising from the *cis*-alkene and having m/z 138 were assigned as 2 (98%), 4 (1%) and, tentatively, the ene product[12] **6** (1%);



6 **7**

the corresponding values for the trans isomer were 2 (1%), **4** (98%), and **6** (1%). Within experimental error, estimated at ±1%, and given the origin of **4**, the cycloadducts **2** and **3** are formed at least 99% stereospecifically.

These stereochemical results require that the mechanism of the [2 + 2] cycloaddition either be concerted or involve biradical or zwitterionic intermediates, e.g., **7**, having lifetimes that are short relative to rotation about a carbon–carbon bond. Obviously the former option would necessitate that cyclopentyne participate in an *antarafacial* sense or that it react through a very low-lying S_1 state, a possibility we consider remote,[13] to avoid violation of the tenets of orbital symmetry.[9] Data that bear on the viability of intermediates being sufficiently short-lived to accommodate the high stereoselectivity are scant. However, to the extent that such an intermediate could be compared to those postulated to account for [2 + 2] cycloadducts from benzyne and alkenes, a lifetime such as to allow substantial stereorandomization would be anticipated.[12] Consequently antarafacial participation of cyclopentyne in a concerted cycloaddition is the mechanism presently favored.[15]

Acknowledgment. The financial support of the National Institutes of Health (GM 29972) and of the Robert A. Welch

(1) Gilbert, J. C.; Baze, M. E. *J. Am. Chem. Soc.* **1983**, *105*, 664.

(2) Strictly speaking, the results of our labeling study are consistent with either cyclopentyne or equilibrating species that have a composite symmetry equivalent to that of cyclopentyne. MINDO/3 calculations suggest that cyclopentyne is a *transition state* linking two π-complexes (eq i).[3]

(i)

(3) Gilbert, J. C., unpublished results.

(4) Fitjer, L.; Kliebisch, U.; Wehle, D.; Modoressi, S. *Tetrahedron Lett.* **1982**, *23*, 1661.

(5) Charles, S. W.; Cullen, F. C.; Owen, N. L. *J. Mol. Struct.* **1973**, *18*, 183.

(6) Analysis of the cis isomer by GLPC showed it to be contaminated with 2% of the trans material; the opposite was found for the trans isomer.

(7) Compound **2**: ^1H NMR (200 MHz, $CDCl_3$) 1.32 (3 H, d, J = 7.0 Hz), 1.8–2.5 (6 H, m), 2.9–3.1 (1 H, m), 3.21 (3 H, s), 4.2 (1 H, m); ^{13}C NMR (22.6 MHz, neat, microcell) 13.94, 25.82, 28.16, 29.07, 40.78, 54.69, 77.00, 151.46, 157.58; HRMS, m/z calcd for $C_9H_{14}O$ 138.104 46, found 138.104 11. Compound **5**: ^1H NMR (200 MHz, $CDCl_3$) 1.52 (3 H, d, J = 7.5 Hz), 2.2–2.7 (5 H, m), 3.03 (3 H, s), 3.23 (1 H, m), 3.33 (1 H, m), 3.98 (1 H, m), 7.1–7.3 (2 H, m), 11.18 (1 H, s), 11.92 (1 H, s); HRMS, m/z calcd for $C_{19}H_{18}O_5$ (molecular ion not observable) 326.115 41, found 326.116 11.

(8) Wasserman, H. H.; Solodar, A. J.; Keller, L. S. *Tetrahedron Lett.* **1968**, 5597. Snider, B. B.; Rodini, D. J.; Conn, R. S. E.; Sealfor, S. *J. Am. Chem. Soc.* **1979**, *101*, 5283.

(9) Hoffmann, R.; Woodward, R. B. *J. Am. Chem. Soc.* **1965**, *87*, 2046.

(10) Vicinal trans alkoxy and methyl groups are expected to lower the activation barrier to ring opening of a monocyclic cyclobutene by about 10 kcal/mol relative to that of cyclobutene itself; a cis relationship would give a decrease of 6.5 kcal/mol, assuming the alkoxy group moves outward.[11] Greater thermal instability of **3** compared to **2** is thus to be expected.

(11) These substituent effects are those obtained by W. Kirmse et al. We thank Prof. Kirmse for providing us with these data.

(12) Jones, M. J., Jr.; Levin, R. H. *J. Am. Chem. Soc.* **1969**, *91*, 6411. Gassman, P. G.; Benecke, H. P. *Tetrahedron Lett.* **1969**, 1089. And references cited in these two sources.

(13) Among other reasons for this belief is the finding that the symmetrical orbital of *trans,trans*-1,5-dehydropentadienyl (i) is lower in energy than the

i

antisymmetrical orbital.[14] These orbitals correspond to the S_0 and S_1 states, respectively, of cyclopentyne. MINDO/3 calculations on cyclopentyne itself yield a similar prediction.[3]

(14) Hoffmann, R.; Imamura, A.; Hehre, W. J. *J. Am. Chem. Soc.* **1968**, *90*, 1499.

(15) After submission of this paper, a report by Fitjer and Modaressi appeared describing results analogous to ours when *cis*- and *trans*-2-butenes were used to trap cyclopentyne.[16] They rationalize the stereospecificity of the cycloaddition as resulting from a ground electronic state of the cycloalkyne that is antisymmetrical, an interpretation that is at variance with ours. Because a methyl as compared to a methoxy group is less able to stabilize a biradical or zwitterionic intermediate and thus is less likely to allow for stereorandomization, the present example represents a more sensitive probe of the mechanism of [2 + 2] cycloaddition of cyclopentyne.

(16) Fitjer, L.; Modaressi, S. *Tetrahedron* Lettt. **1983**, *24*, 5495.

0002-7863/84/1506-1885$01.50/0

Foundation (F-815) for this research is gratefully acknowledged. We thank Dr. Steven Larson for the X-ray crystallographic results and Kevin Sweeney for GC-MS analyses.

Supplementary Material Available: ORTEP structure, crystallographic data, and bond lengths and angles for adduct **5** (3 pages). Ordering information is given on any current masthead page.

2′-Azido-2′-deoxynucleotide Interaction with *E. coli* Ribonucleotide Reductase: Generation of a New Radical Species

M. Ator, S. P. Salowe, and J. Stubbe*†

Biochemistry Department
College of Agricultural and Life Sciences
University of Wisconsin—Madison
Madison, Wisconsin 53706

M. H. Emptage

Enzyme Institute, University of Wisconsin—Madison
Madison, Wisconsin 53705

M. J. Robins

Chemistry Department, University of Alberta
Edmonton, Alberta, Canada

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Ribonucleotide reductase (RDPR)[1] from *E. coli* catalyzes the conversion of nucleoside diphosphates to deoxynucleoside diphosphates.[2] This enzyme is composed of two subunits: B_1 (α, α' M_r 160000) binds NDP[1] substrates and contains redox active thiols and binding sites for the allosteric effectors; B_2 (β,β M_r 78000) contains an unusual cofactor composed of two Fe^{+3} and one tyrosine radical, which is an integral part of the B_2 polypeptide chain. The active site is thought to be at the interface between the two subunits.[2] Evidence from our laboratory is consistent with a proposed radical cation mechanism for this reduction reaction.[3] However, until recently, no direct evidence in support of any substrate radical intermediates was available.[4] Sjöberg et al. observed that incubation of RDPR with suicide inhibitor 2′-azido-2′-deoxycytidine 5′-diphosphate (N_3CDP)[1] resulted in the formation of a new radical species.[4] Furthermore in the presence of ^{15}N- or ^{2}H-labeled RDPR and H_2O or D_2O the new radical generated showed no change in its hyperfine splitting pattern. They interpreted this data as evidence for formation of a "substrate analogue" radical. These studies prompted us to report our findings with specifically labeled substrate analogues [2′-^{2}H]N_3UDP[1] and [2′-^{15}N]N_3UDP. Our results clearly indicate formation of the same radical species as observed by Sjöberg et al. Results with the [2′-^{15}N]N_3UDP and [2′-^{2}H]N_3UDP indicate that the new radical is indeed located on a nitrogen originally at the 2′-position of the substrate and that the observed coupling of this species to hydrogen is not caused by the hydrogen on the 2′-carbon. Structures proposed by Sjöberg et al. for this radical are inconsistent with these results.

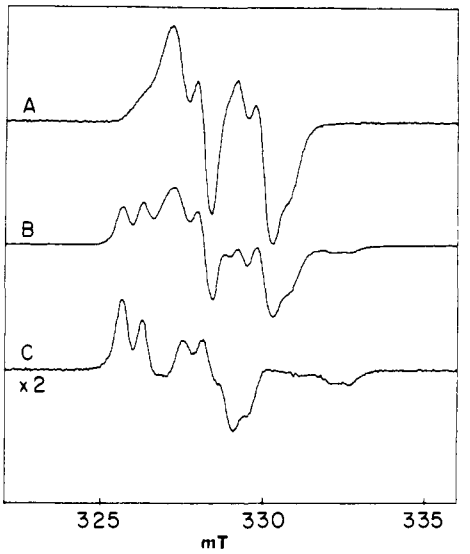

Figure 1. EPR spectra of RDPR with N_3UDP: (A) RDPR in the absence of N_3UDP, (B) 7 min after the addition of N_3UDP, (C) after subtraction of the remaining tyrosine radical signal A from B. Spectrometer conditions: microwave frequency, 9.224 GHz; microwave power, 10 μW; modulation amplitude, 0.2 mT; temperature, 13 K; scanning rate, 16 mT/min; time constant, 0.0645.

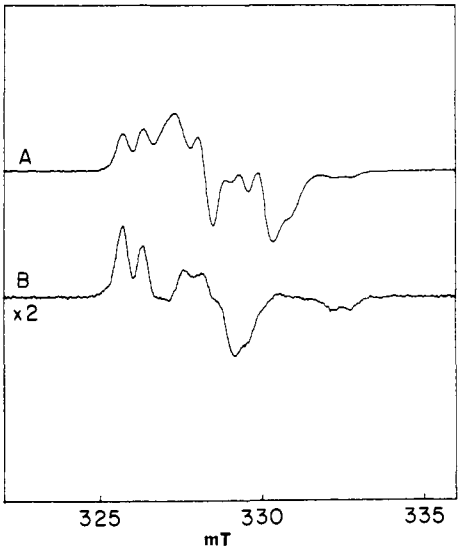

Figure 2. EPR spectra of RDPR with [2′-^{2}H]N_3UDP: (A) 7 min after the addition of [2′-^{2}H]N_3UDP to RDPR, (B) after subtraction of the remaining tyrosine radical signal from A. Spectrometer conditions are as in Figure 1.

Incubation of 17.6 nmol of B_2 under standard assay conditions[5] followed by freezing in liquid N_2 resulted in the EPR spectrum of the tyrosine radical observed in Figure 1A. The sample was then thawed and equilibrated at 25 °C, and N_3UDP (final concentration of 1.5 mM) was added. The reaction was allowed to proceed for 7 min at 25 °C and the sample again frozen in liquid N_2,[6] resulting in the spectrum indicated in Figure 1B. Figure 1C is the spectrum of the new radical species after subtraction of the remaining tyrosine radical spectrum (Figure 1A) from the spectrum of Figure 1B. This species is essentially identical with that observed by Sjöberg et al. with N_3CDP.[4] The hyperfine

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(1) Abbreviations: RDPR, ribonucleoside diphosphate reductase; N_3NDP, 2′-azido-2′-deoxynucleoside 5′-diphosphate; NDP, nucleoside diphosphate; mT, millitesla.

(2) For recent reviews: Thelander, L.; Reichard, P. *Annu. Rev. Biochem.* **1979**, *48*, 143. Reichard, P.; Ehrenberg, A. *Science (Washington, D.C.)* **1983**, 221, 514.

(3) Stubbe, J. A.; Ackles, D. *J. Biol. Chem.* **1983**, *255*, 8027. Stubbe, J. A; Ator, M; Krenitsky, T. *Ibid.* **1983**, *258*, 1625.

(4) Sjöberg, B.-M.; Gräslund, A.; Eckstein, F. *J. Biol. Chem.* **1983**, *258*, 8060.

(5) All EPR spectra were run in D_2O. Proteins were exchanged into D_2O by centrifugation through a 1-mL column of Sephadex G-25 with equilibrated HEPES (pD 7.2), 15 mM $MgSO_4$, 1 mM EDTA in D_2O. Typical reaction mixtures contained in a final volume of 0.3 mL: 50 mM HEPES (pD 7.2), 15 mM $MgSO_4$, 1 mM EDTA, 90 μm TTP, 0.5 mM NADPH, 0.3 mg of thioredoxin, 0.05 mg of thioredoxin reductase, 1.4 mg (17.6 nmol) of B_2, and 1.1 mg (7 nmol) of B_1.

(6) A time course of radical formation with N_3UDP derivatives showed maximum radical formation at about 7 min.

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