dicyanoacetylene and diethyl azodicarboxylate, we have been able to isolate only one further bisadduct. It is formed by using the potent *N*-phenyltriazolinedione (**8**) as dienophile, and its nmr spectrum again shows two sharp methyl singlets, consistent with the assignment of its structure as **7b**.

Fortunately, **8** is such a reactive dienophile that the rate of the initial Diels–Alder reaction is competitive with rate of rearrangement of **3b**. When the reaction of **2** with 1 equiv of **8** is conducted at 0° in chloroform, a monoadduct can be isolated by chromatography. Its nmr spectrum is wholly consistent with structure **6b** and excludes **4b**. In particular, the methyl group which resonates at lowest field (δ 1.51, $CDCl_3$) is coupled with $J = 2$ Hz to a proton which appears centered at δ 3.71 as half of an AB quartet. The chemical shift and allylic coupling are indicative of a methyl group on a double bond. Since in **4b** none of the methyl groups are located on double bonds, the nmr spectrum demands structure **6b**. Compound **6b** reacts with another equivalent of **8** to give **7b**.

The rapidity of the initial Diels–Alder reaction has enabled us to prepare **3b** at low temperature and measure its rate of rearrangement to **6b**. When equimolar **2** and **8** are mixed in $CDCl_3$ and allowed to warm to +10° in an nmr probe, a reaction occurs as evidenced by the disappearance of the resonances of **2** and the appearance of new singlets at δ 0.67 (6 H), 1.14 (6 H), 4.32 (4 H), 4.85 (2 H), and 5.48 (2 H). When the sample is allowed to warm to 30°, these resonances, ascribed to **3b**, diminish in intensity as those of **6b** become larger. The half-time for the rearrangement is approximately 10 min at this temperature.

In summary, we have shown that a $_{\sigma}2_a + {}_{\sigma}2_s$ mechanism is ruled out in the very rapid rearrangement of an ethylene-bridged cyclobutane. The demonstration of the nonintervention of a transition state of purported special stability suggests that the facility with which such molecules undergo rearrangement results from their high energy content. In addition to the large amount of strain presumably present in a cyclobutane ring 1,3 bridged by ethylene,[15] unfavorable π interaction between the orbitals of the ring and bridge also contributes to the instability of molecules containing this moiety.[17]

(15) The unusually long carbon–carbon bonds present in tricyclo-[3.3.0.0^{2,6}]octene[16] give evidence of the highly strained nature of this molecule.

(16) D. L. Zebelman, S. H. Bauer, and J. F. Chiang, *Tetrahedron*, 28, 2727 (1972).

(17) In cyclobutane rings 1,3 bridged by ethylene the highest occupied molecular orbitals (HOMO's) of the ring and the bridge have the same symmetry and consequently mix.[18] Interaction between such filled orbitals is shown to be destabilizing by calculations which include overlap.[8] Although stabilizing interactions between occupied and unoccupied orbitals—for instance, between the second highest filled MO of the ring and π^* of the bridge—do occur, they are cancelled by the "overlap repulsion" between the HOMO's.

In contrast, the orbital interactions in cyclobutane 1,3 bridged by butadiene result in net stabilization, because the symmetries of the HOMO and LUMO in butadiene are the reverse of those in ethylene. We attribute the reluctance of **2** and **3** to undergo Diels–Alder reactions, at least in part,[19] to the favorable orbital interactions between ring and bridge when butadiene, rather than ethylene, is the bridging group.

A fuller discussion than that possible here will be published along with the results of calculations that have been carried out in this laboratory by W. L. Jorgenson and which support these conclusions.

(18) R. Hoffmann and R. B. Davidson, *J. Amer. Chem. Soc.*, 93, 5699 (1971). The authors have suggested that interaction between these orbitals is responsible for the unusual uv spectrum of **1**. The effect of this mixing on the total energy of the molecule is implicit in their interaction diagram.

Acknowledgment. We wish to thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the National Science Foundation for support of this work. We also thank Professor Jean-Marie Lehn and Dr. G. Wipff for making available to us some preliminary results of their calculations on bridged cyclobutanes.

(19) The shorter bond in an ethylene bridge (1.34 *vs.* 1.48 Å in butadiene) probably results in a more strained system; therefore, strain may also contribute to the energetic preference for butadiene as the 1,3-cyclobutane bridging group.

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The Reaction of Oxazoles with Singlet Oxygen. The Mechanism of the Rearrangement of Triamides

Sir:

Among the unusual transformations observed in the reaction of singlet oxygen with organic substrates has been the facile conversion of oxazole derivatives to triamides.[1-3] This remarkable rearrangement takes place in high yields under mild conditions when the excited oxygen is generated both by chemical[4] and photooxidative methods. We now wish to report tracer studies which clarify the mechanism of this conversion.

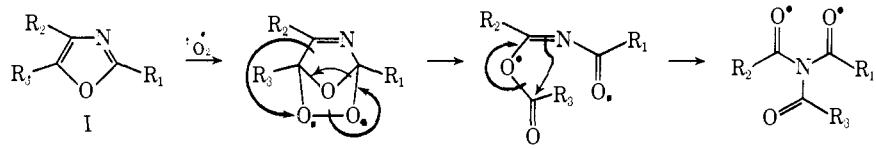
We had suggested earlier that the oxidative change takes place by initial formation of a transannular peroxide, followed by a Baeyer–Villiger-like rearrangement to an intermediate imino anhydride, Chart I, path a. An *O*-acyl to *N*-acyl migration would then lead to the triamide. The ease with which imino anhydrides rearrange to triamides is well known.[5]

A very reasonable alternate route to an intermediate imino anhydride (path b) would involve addition of oxygen at the 4,5 position of the oxazole followed by cleavage of the dioxetane ring. This (isomeric) imino anhydride could then rearrange, as described above, to form the triamide.

There are many precedents for both types of singlet oxygen reaction with heterocyclic systems. In the furan series, oxygen insertion products of the Baeyer-Villiger type have been commonly observed,[6-8] while with substituted imidazoles and imidazolones ring cleavage through dioxetanes appears to be the predominant reaction course.[9,10] In other highly electron-rich unsaturated systems, stable intermediate dioxetanes have been isolated.[11-13]

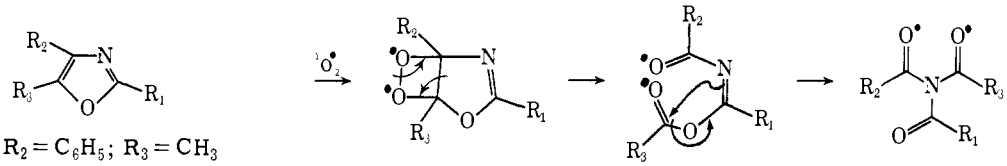
(1) H. H. Wasserman and M. B. Floyd, *Tetrahedron, Suppl.*, No. 7, 441 (1966).
(2) H. H. Wasserman and E. Druckrey, *J. Amer. Chem. Soc.*, 90, 2440 (1968).
(3) H. H. Wasserman, *Ann. N. Y. Acad. Sci.*, 171, 108 (1970).
(4) H. H. Wasserman, J. R. Scheffer, and J. L. Cooper, *J. Amer. Chem Soc.*, 94, 4991 (1972).
(5) D. Y. Curtin and L. Miller, *Tetrahedron Lett.*, 1869 (1965).
(6) G. O. Schenck, *Justus Liebigs Ann. Chem.*, 584, 156 (1953).
(7) H. H. Wasserman and A. Liberles, *J. Amer. Chem. Soc.*, 82, 2086 (1960).
(8) H. H. Wasserman, A. Doumaux, and R. E. Davis, *ibid.*, 88, 4517 (1966).
(9) T. Matsuura and I. Saito, *Tetrahedron Lett.*, 3273 (1968).
(10) M. L. Wolff, Ph.D. Thesis, Yale University, 1970.
(11) P. D. Bartlett and A. D. Schaap, *J. Amer. Chem. Soc.*, 92, 3223 (1970).
(12) S. Mazur and C. S. Foote, *ibid.*, 92, 3225 (1970).
(13) K. R. Kopecky and C. Mumford, *Can. J. Chem.*, 47, 709 (1969).

Chart I[a]



I

path a



I_x, $R_1 = R_2 = C_6H_5$; $R_3 = CH_3$
I_y, $R_1 = CH_3$; $R_2 = R_3 = C_6H_5$
I_z, $R_1 = R_2 = CH_3$; $R_3 = p\text{-}NO_2C_6H_4$

path b

[a] Oxygen labeled with a solid circle represents oxygen enriched with ^{18}O.

We sought to distinguish between the two mechanisms of oxygen addition and decomposition by allowing the oxazoles to react with singlet oxygen enriched with oxygen-18. A particularly convenient method of introducing the labeled singlet oxygen into the medium involved the use of 9,10-diphenylanthracene peroxide as an oxygen-18 donor.[4,14,15]

The oxidations were carried out[4] using three representative oxazoles (Chart I) and the products isolated as previously described.[1,16] The location of the labeled oxygen was determined by analysis of the fragmentation patterns of the triamides using high-resolution mass spectroscopy.[17]

Considering the two alternative pathways a and b for the oxidation of I_x ($R_1 = R_2 = C_6H_5$; $R_3 = CH_3$) it is clear that path a leads to a triamide in which the enriched oxygen atoms are both located in benzoyl residues. Path b, on the other hand, leads to a triamide in which the labeled oxygen atoms are distributed between the acetyl and the benzoyl groups.

Experimentally it was found that in the oxidation of I_x all of the enriched oxygen was located in the benzoyl groups. The parent mass spectral peak appeared at *m/e* 267 along with a P + 4 peak at 271 indicating the incorporation of two atoms of oxygen-18. The extent of double incorporation was 8.4%.[18] A peak at *m/e* 225 due to the loss of an acetyl residue is accompanied by a 229 (P + 4) peak (8.1%)[18] clearly showing that the acetyl group does not contain the labeled oxygen. In the same spectrum one observes a benzoyl peak at *m/e* 105 accompanied by the expected P + 2 peak at 107 (8.0%).[18]

The above results establish the location of the enriched oxygen in the R_1CO and R_2CO residues and are clearly in accord with path a oxidation. An oxidation involving path b would have distributed the labeled oxygen into the R_2CO and R_3CO fragments, showing a 227 (P + 2) instead of a 229 enrichment peak. The peak at *m/e* 107 would, at the same time, be reduced in intensity to *ca.* 4.2%[18] due to dilution by an unlabeled benzoyl group.

Further evidence in support of the path a hypothesis was provided by analysis of the triamides derived from oxidations of oxazoles I_y and I_z. The mass spectrum of the *N*-acetyldibenzamide derived from I_y shows the peaks and the enrichment values expected from path a, but not path b. In particular, the peak at *m/e* 107 corresponding to enriched benzoyl is only 4.5%[18] in intensity in accord with the expected dilution by one unlabeled benzoyl group. In the case of oxazole I_z no parent peak was observed. However, the peak due to the loss of acetyl (*m/e* 208) is accompanied by a P + 2 peak at 210 (8.9%[18] enrichment) showing that the acetyl residue which is lost contains one of the oxygen-18 atoms.

In a separate series of experiments in which the labeled oxygen was incorporated into the oxazole ring it was again clearly shown that path a rather than path b oxidation is involved in the oxazole–triamide conversion. Compounds II and III were prepared by standard means[19] using acetamide enriched with oxygen-18. Dye-sensitized photooxygenation in methanol using unenriched (O-16) oxygen yielded the products shown in Chart II. As predicted by the path a mechanism all of the isotopic enrichment was observed in the *p*-nitrobenzoyl and benzoyl fragments, respectively, and none in the acetyl groups.

(14) H. H. Wasserman and J. R. Scheffer, *J. Amer. Chem. Soc.*, **89**, 3073 (1967).

(15) For the preparation of the labeled peroxide, 9,10-diphenylanthracene (1.50 g) in 700 ml of spectrograde CS_2 was irradiated with a GE 200-W (Quartzline) immersion lamp. The system, purged with nitrogen, contained 100 ml of $^{16}O_2$ and 100 ml of 90% enriched $^{18}O_2$ (Miles Laboratories). Water cooling kept the solution temperature below 25°, and O_2 uptake was measured quantitatively. When the theoretical amount of oxygen had been consumed, the reaction was stopped. After removal of polymeric impurities and recrystallization, 1.08 g of 38.0% doubly labeled 9,10-diphenylanthracene peroxide was obtained.

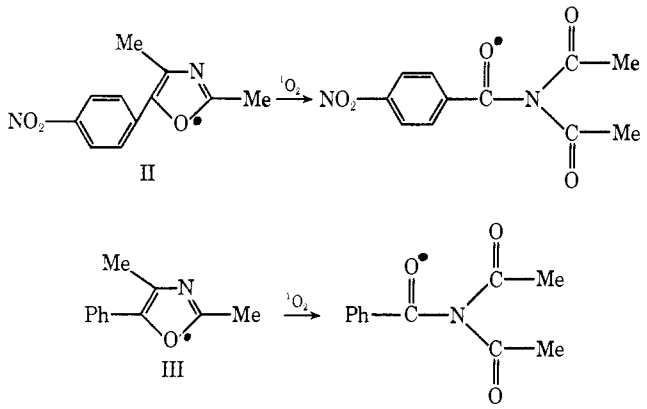
(16) A typical oxidation with labeled 9,10-diphenylanthracene peroxide was carried out as follows. The labeled peroxide was diluted with unlabeled material to approximately 12% double enrichment. The labeled peroxide (120 mg) and the oxazole (60 mg) were warmed to reflux in dry benzene (10 ml) until a tlc analysis showed complete consumption of starting material. The solvent was evaporated *in vacuo* and the residue chromatographed on silica gel yielding pure triamide (80–90%) (1:1 benzene–hexane).

(17) The mass spectrometric determinations were performed by Dr. Walter McMurray, Division of Physical Sciences, Yale University, using an AEI MS-9 double-focusing mass spectrometer. The spectra were recorded and processed to give masses and relative abundances on an IBM 1800 computer.

(18) Percentage values represent the intensities of the ^{18}O P + 2 or P + 4 peaks (above natural isotopic abundance) relative to the peaks containing only ^{16}O, normalized to 100%. Reported values are accurate to ±0.5%.

(19) B. S. Friedman, M. Sparks, and R. Adams, *J. Amer. Chem. Soc.*, **59**, 2262 (1937).

Chart II



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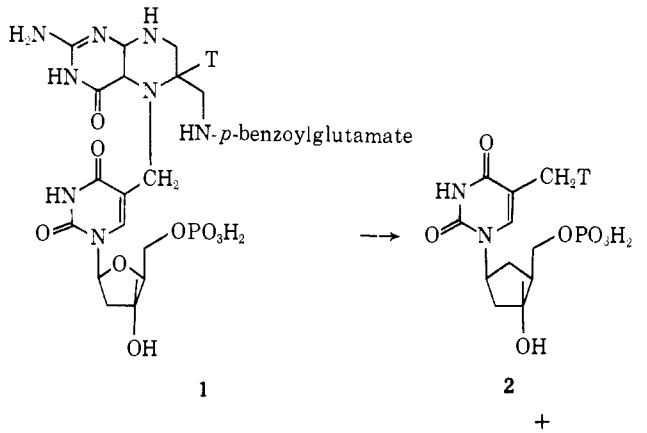
A Chemical Model for Thymidylate Synthetase Catalysis

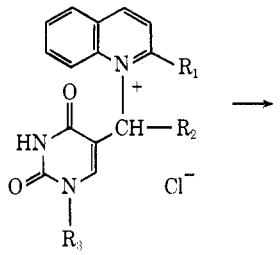
Sir:

The enzyme-catalyzed conversion of 2′-deoxyuridine 5′-phosphate (dUMP) to thymidine 5′-phosphate (**2**, TMP), a reductive methylation, utilizes formaldehyde as the carbon source and tetrahydrofolic acid (THFA) as the reducing agent.[1] In a definitive study of the mechanism of this enzymatic reaction Wahba and Friedkin proposed structure **1** as an intermediate. From their observations on the transfer of tritium from C-6 of THFA to the methyl group of TMP they suggested that **1** undergoes a 1,3 hydride shift. However, Gupta and Huennekins[2] reported that an analog of **1**, 5-thyminyltetrahydrofolic acid, was stable in air and did not undergo rearrangement to thymine when heated to 100° at pH 7.

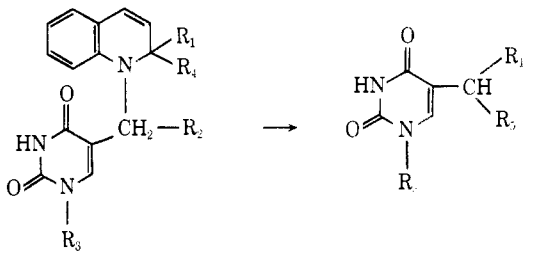


1 **2** + 7,8-dihydrofolic acid

This communication describes a chemical model in support of Wahba and Friedkin's mechanism for methylation and reduction leading to TMP catalyzed by the title enzyme. Treatment of 5-chloromethyluracil[3] with quinoline gave *N*-thyminylquinolinium chloride (**3a**, mp 278–280).[4] Reduction of **3a** with



3a, $R_1 = R_2 = R_3 = H$
3b, $R_1 = R_2 = H$; $R_3 = CH_3$
3c, $R_2 = R_3 = H$; $R_1 = {}^2H$
3d, $R_1 = R_3 = H$; $R_2 = {}^2H$



4a, $R_1 = R_2 = R_3 = R_4 = H$
4b, $R_1 = R_2 = R_4 = H$; $R_3 = CH_3$
4c, $R_2 = R_3 = H$; $R_1 = R_4 = {}^2H$
4d, $R_1 = R_3 = R_4 = H$; $R_2 = {}^2H$

5a, $R_1 = R_2 = R_5 = H$
5b, $R_1 = R_5 = H$; $R_3 = CH_3$
5c, $R_1 = R_3 = H$; $R_5 = {}^2H$

$NaBH_4$ gave 1,2-dihydro-*N*-thyminylquinoline (**4a**, mp 190–205°, mass spectral peak matching: calcd 255.10069; found 255.10078.). Heating a neat sample of **4a** to 205° resulted in rearrangement to give quinoline (49%) and thymine (**5a**, 42%).[5] Alternatively **4a** was rearranged to give **5a** (24%) by refluxing in diglyme 90 min or a 3% yield of **5a** upon 4-hr reflux of an aqueous solution of **4a**. Another analog, 1,2-dihydro-*N*-(1-methylthyminyl)quinoline (**4b**, mp 155–160°), prepared by the same method as **4a** gave 5% of 1-methylthymine (**5b**).

Quinoline-*2-d*, prepared by decarboxylation of quinaldic acid–COOD, was used for the preparation of **3c** (mp 277–278°); $NaBD_4$ reduction of **3c** gave 1,2-dihydro-*N*-thyminylquinoline-*2,2-d*$_2$ (**4c**, mp 217–223°; nmr (DMSO-d_6) δ 7.2 (s, 1 H, uracil C_6H), 3.85 (s, 2 H, NCH_2 uracil). Refluxing either a 50% dioxane solution or a diglyme solution of **4c** for 48 hr gave, respectively, an 18 and 34% yield of thymine-*methyl-d* (**5c**, mp 280–285°, nmr (DMSO-d_6) δ 1.75 (s, 2 H, uracil CH_2D); mass spectrum 70 eV *m/e* (relative intensity) 128 (13), 127 (100), 126 (13)). 1,2-Dihydro-*N*-thyminyl-*methyl-d*-quinoline (**4d**, mp 190–200°, nmr (DMSO-d_6) δ 3.9 (s, 1 H, NCHD uracil), mass spectrum 70 eV *m/e* 256) was prepared from 5-formyluracil *via* reduction with

(1) (a) A. J. Wahba and M. Friedkin, *J. Biol. Chem.*, **237**, 3794 (1962); (b) M. Friedkin, *Annu. Rev. Biochem.*, **32**, 185 (1963); (c) R. L. Blakely, "The Biochemistry of Folic Acid and Related Pteridines," Wiley, New York, N. Y., 1967.

(2) V. S. Gupta and F. M. Huennekins, *Biochemistry*, **6**, 2168 (1967).

(3) (a) R. E. Cline, R. M. Fink, and K. Fink, *J. Amer. Chem. Soc.*, **81**, 2521 (1959); (b) J. H. Burckhalter, R. J. Siewald, and H. C. Scarborough, *ibid.*, **82**, 991 (1960).

(4) All compounds with the exception of **4c** and **4d** had acceptable carbon, hydrogen, and nitrogen analyses. All compounds had the expected ir, uv, nmr, and mass spectral patterns. Thymine and its derivatives (**5d–c**) were identified by melting point, ir, and tlc.

(5) A substituted 1-methyl-1,2-dihydroquinoline derivative has been reported to yield methane and the substituted quinoline on heating: J. Meisenheimer and M. Schutze, *Ber.*, **56**, 1353 (1923).