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Reactions of urocanic acid (UCA) methyl esters with singlet oxygen and 4-methyl-1,2,4-triazoline-3,5-dione (MTAD)

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Dedicated in memory of Professor Christopher S. Foote

Abstract—Singlet oxygen adds to the imidazole ring of *cis*- and *trans*-methyl urocanate (MUC) to yield the corresponding 2,5-endoperoxides, which are modestly stable at low temperature but decompose upon warming to form complex reaction mixtures. MTAD, a singlet oxygen mimic, reacts with *cis*- and *trans*-MUC to yield stereospecific [4+2] reaction products involving the olefinic side chain and the C_4–C_5 double bond of the imidazole ring. *trans*-MUC forms a 1:2 MTAD adduct while the cis isomer yields only the 1:1 adduct at 25 °C. The stereospecificity and absence of MeOH trapping adducts indicate that these reactions may not involve open or trappable dipolar intermediates.
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1. Introduction

3-(1*H*-Imidazol-4(5)-yl)-2-propenoic acid commonly referred to as urocanic acid (UCA) is a metabolite of histidine and is present in the stratum corneum of the human skin. UCA, produced naturally as the trans isomer, accumulates in the upper layers of the epidermis and isomerizes to *c*-UCA upon absorption of UV light[1,2] (Fig. 1). *t*-UCA was found to absorb ultraviolet B light and thus considered to be a natural sunscreen against harmful UV rays. This led to the addition of UCA to sunscreens and skin lotions to help prevent skin cancer and skin related diseases. However, subsequent studies indicated that *t*-UCA can act as an initiator of immunosuppression, which may be critical to UV-induced pathogenesis of skin cancer and other cutaneous diseases.[3,4]

It was recently reported that photoexcited *t*-UCA can lead to the formation of reactive oxygen species, which are linked to a number of diseases and disorders, i.e., enzyme inactivation, mutations, premature aging, DNA damage, and respiratory problems.[5] The oxidative products of UCA are proposed to be responsible for UV-induced immune suppression. In 2002, Elton and Morrison confirmed that the irradiation of UCA with monochromatic light at 351 nm produces singlet oxygen.[6] They concluded that UCA is a better scavenger than generator of singlet oxygen and may play an important role as an antioxidant. Colorimetric tests (EM Quant peroxide strip) on the reaction products of singlet oxygen with UCA showed the presence of peroxides,[6] but detailed product studies have not been reported. UCA-singlet oxygen products catalyze further photo-destruction of UCA and UCA peroxides can be major contributors to photo-initiated nicking of plasmid DNA.[6]

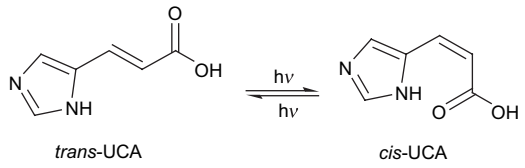

Figure 1. Photoisomerization of UCA.

The reactions of singlet oxygen with imidazole ring containing systems, especially histidine and guanine analogs, have received considerable attention. Early studies on the photooxygenation of histidine demonstrated that complex reaction mixtures are produced, i.e., the reaction of singlet oxygen with *N*-benzoyl histidine leads to the formation of 17 products.[7] Wasserman and Lipshutz conducted extensive studies on the reactions of singlet oxygen with substituted imidazoles, proposed the involvement of 2,5-endoperoxides, and reported two main pathways of oxidation depending on the substitution pattern of the imidazole ring.[8] When the imidazole ring bears a hydrogen atom at the 2- and/or 5-position, a carbonyl group is produced at that specific site. In the absence of a proton at the 2- or 5-position the formation of the dioxetane by [2+2] addition of singlet oxygen to the C4–C5 bond in the imidazole appears to be operative. Ryang and Foote later reported the direct observation of imidazole 2,5-endoperoxides as products of singlet oxygen.[9]

Keywords: Photooxygenation; Singlet oxygen; Endoperoxides; Urocanic acid.
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Singlet oxygenation of guanine and its nucleosides (guanosine) have received considerable attention because of the implications on the function of DNA and its importance during photodynamic therapy (PDT) used for the treatment of malignant tumors.[10,11] Because of the limited solubility of guanine substrates, the instability of intermediates and complexity of the product mixtures, the mechanism of photosensitized oxidation of guanine and its nucleosides was unclear until recently.[11] Foote and co-workers used a variety of substituted guanosine adducts, isotopic labeling, and low temperature NMR to suggest that singlet oxygenation of guanosine involves a series of reactive intermediates, including endoperoxides, hydroperoxides, and dioxiranes.[11b,c] The reaction pathways are highly sensitive to solvent effects and the substitution pattern, i.e., only the C-8 methylated substrate reacts to form an observable endoperoxide, which collapses to yield singlet oxygen and the starting substrate (Fig. 2). Kang and Foote conducted detailed product studies on isotopically labeled imidazole ring systems and demonstrated that the reaction pathways are dramatically influenced by N-alkylation of the parent compound[12] (Fig. 2).

The photooxidation of UCA may be a main pathway for premature aging, UV-induced immune suppression, and skin cancer,[13,14] yet the mechanisms and products of photooxidation have not been clearly established. While previous studies on the singlet oxygenation of imidazole ring systems provide tremendous insight about the possible reaction pathways of singlet oxygen with UCA, the presence of the conjugated enone side chain in UCA may lead to different singlet oxygen reactivities.

Because of the insoluble nature of UCA in organic solvents, the methyl ester, MUC, was used as a model for UCA. MUC is modestly soluble in the organic solvents required for low temperature NMR studies. MUC is expected to exhibit the same reactivity as UCA toward singlet oxygen. We report herein singlet oxygenation of *cis*- and *trans*-MUC yields 2,5-endoperoxides, which are modestly stable at low temperature but decompose at room temperature to afford complex product mixtures. Given the unstable nature of the endoperoxides and the complexity of the reaction product mixtures we also studied the reactions of MUC with MTAD, a singlet oxygen mimic. Surprisingly, MTAD and singlet oxygen exhibit dramatically different reactivities toward MUC.

2. Results and discussion

2.1. Reactions of singlet oxygen with MUC

Since the solubility of UCA in organic solvents is too low to conduct detailed NMR studies, *trans*-UCA was converted to the corresponding methyl, *n*-butyl, and *tert*-butyl esters, using acid promoted esterification. *t*-MUC showed the best solubility among the esters in the organic solvents commonly used for low temperature NMR. *t*-MUC is easily isomerized to *c*-MUC by irradiation with 254 nm light under argon-saturated conditions.[15] The cis isomer was purified using standard flash column chromatography.

For the typical experiment, MUC was dissolved in 0.5–1 mL of the appropriate NMR solvent (CD_2Cl_2, acetone-d_6, CD_3CN, or $CDCl_3$) along with Rose bengal, the singlet oxygen sensitizer. The solution was transferred to a 5 mm NMR tube and placed in ice water in a windowed Dewar. The sample was gently purged with oxygen and irradiated using 200 W Hg–Xe lamp. Pyrex glass was employed to filter light $\leq$320 nm. Control experiments showed no conversion of the *cis*- or *trans*-MUC in the absence of oxygen (argon saturated) or by direct photolysis (in the absence of Rose bengal). The photosensitized oxidation of *t*-MUC, monitored by ^{1}H NMR, was complete within 5 min and continued irradiation did not change the product distribution.

Likely pathways for the reaction of the MUC with singlet oxygen are illustrated below (Fig. 3). They include [4+2] cycloaddition to the imidazole ring to form a 2,5-endoperoxide **1**, [4+2] across the double bond of the side chain

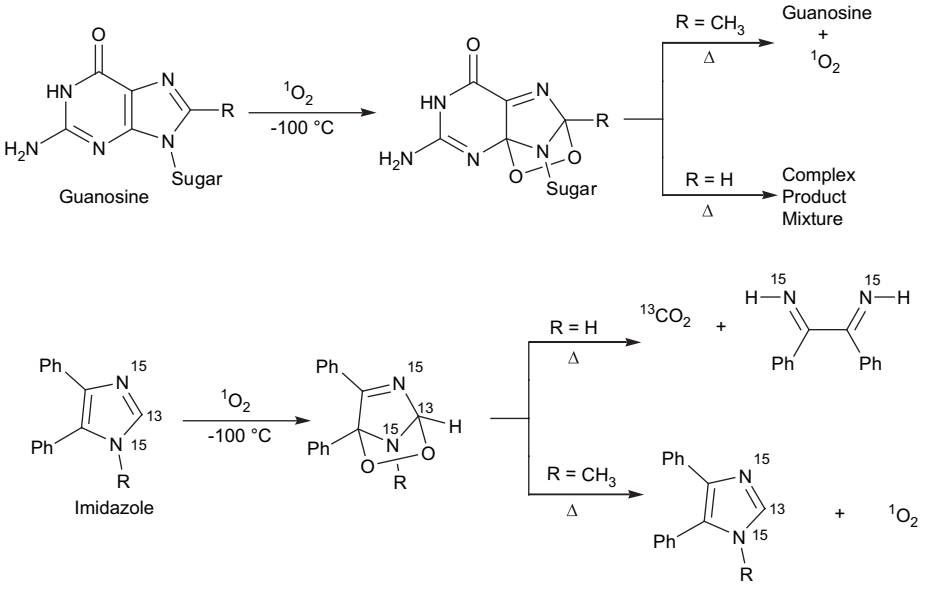

Figure 2. Singlet oxygenation products of guanosine[11] and labeled imidazole.[12]

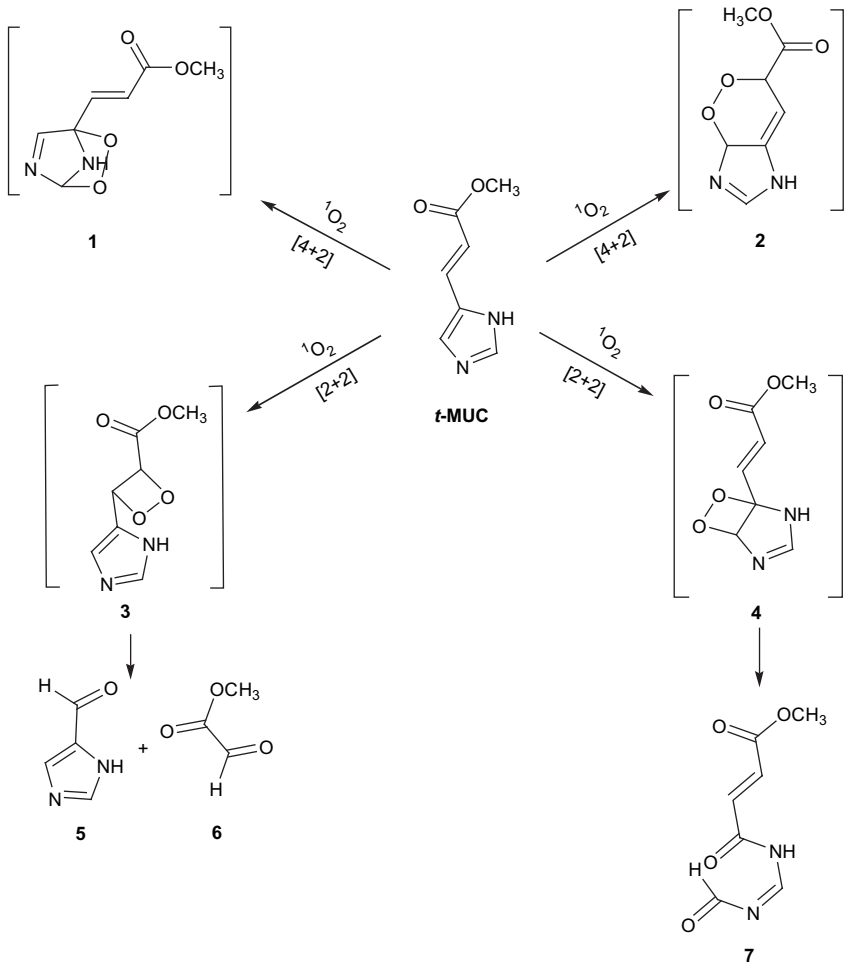

Figure 3. Likely pathways for the reaction of singlet oxygen with *t*-MUC.

and the C_4–C_5 double bond of the imidazole ring to yield endoperoxide **2**, and [2+2] cycloadditions to the side chain to form dioxetane **3** or across the C_4–C_5 double bond of the ring to form **6**. Dioxetanes generally collapse to the corresponding carbonyl compounds at room temperature.

The reaction products of singlet oxygen and *t*-MUC at room temperature exhibit a nearly continuous series of overlapping ^{1}H NMR peaks between 6 and 8 ppm. The complex nature of the ^{1}H NMR spectrum indicates a considerable number of products formed at room temperature. The [2+2] addition to the side chain would result in the formation of the dioxetane **3**, which will collapse to **5** and **6**. ^{1}H NMR and GC–MS analyses of the reaction solution show no evidence of **5** or **6**, thus indicating the [2+2] reaction does not occur at the side chain of *t*-MUC under our experimental conditions. The room temperature ^{1}H NMR spectrum also does not correspond to endoperoxide **1** in accordance with chemical shifts reported for analogous compounds.[12] To test for the formation of endoperoxide **2** we subjected the reaction mixture at low temperature to treatment with cobalt(II) tetraphenylporphyrin, which is known to convert endoperoxides to furans via loss of water.[16] Subsequent ^{1}H NMR and GC–MS analyses showed no evidence that the corresponding furan was formed. These results indicate that singlet oxygen does not react at the side chain of *t*-MUC.

Given the complex nature of the product mixtures observed at room temperature and the fact that endoperoxides and dioxetanes often decompose at room temperature the photooxygenation and ^{1}H NMR characterization were conducted at low temperature. The NMR tube containing the reaction solution was kept at −78 °C throughout the photooxygenation (dry ice/acetone bath in a windowed Dewar). The NMR tube was transferred to a pre-cooled NMR magnet and the proton spectrum collected at −78 °C. The solution was gradually warmed within the NMR magnet and proton spectra collected at −40, −20, 0, and 25 °C. Dramatic differences were observed in the spectra obtained at 0 and 25 °C. The predominant peaks in the low temperature ^{1}H NMR spectrum are singlets at 8.02 and 4.90 ppm (1H/ea); a pair of doublets at 6.80 (1H) and 6.51 ppm (1H) with J=16.1 Hz indicating the trans double bond is not oxidized; and a methoxy resonance at 3.63 ppm. While solubility limitations at low temperatures did not allow for acquisition of a carbon NMR spectrum, the proton spectrum for the reaction mixture at low temperature is consistent with **1** *trans* based on the literature values for the endoperoxide of 1-methylimidazole (Fig. 4). There are no peaks indicating the presence of dioxetane products down to −78 °C.

Singlet oxygenation of *c*-MUC was also conducted at low temperature. The ^{1}H NMR of the product is shown below (Fig. 5). The characteristic ^{1}H NMR peaks are as

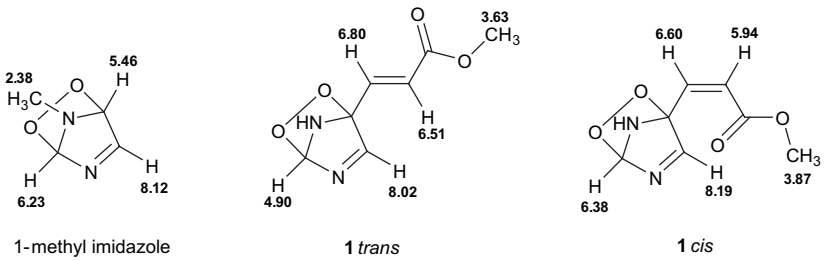

Figure 4. Comparison of the ^{1}H NMR chemical shifts for the 2,5-endoperoxides of 1-methyl imidazole[9] with **1** *trans* and *cis*.

follows: 8.19 (1H) and 6.38 (1H) ppm, a pair of doublets at 6.57 (1H) and 5.94 (1H) ppm with J=11.6 Hz indicating the cis double bond is not oxidized, and a singlet at 3.87 (3H) corresponding to the methoxy resonance. The results are consistent with the formation of the endoperoxide formed by [4+2] addition of singlet oxygen to the imidazole ring.

A number of 2,5-endoperoxides decompose via retro Diels–Alder reaction to yield starting material and singlet oxygen. Tetramethylethylene singlet oxygen trapping experiments indicate the endoperoxides of MUC do not decompose to singlet oxygen upon warming from −78 °C to room temperature. The formation of the endoperoxides can occur by concerted or non-concerted processes.[17] To test for the possible involvement of dipolar intermediates, 3 equiv of methanol was added to the solvent prior to running the reaction. Under these conditions the formation of methanol adducts indicates the involvement of dipolar intermediates.[18,19] The ^{1}H NMR spectra of the reaction run in the presence of MeOH did not indicate the formation of MeOH adducts or any change in the product distribution at −78 and 0 °C. While the presence of MeOH adducts would clearly indicate the involvement of dipolar intermediates, under our experimental conditions, where MeOH (3 equiv) was added to a relatively non-polar solvent a dipolar intermediate may be too short lived to be trapped. There was also no significant change in the product distribution using CD_2Cl_2, acetone-d_6, $CDCl_3$, or CD_3CN as solvents, suggesting that the polar intermediates may not involve in the product forming reaction pathways.

2.2. Reactions of MTAD

Due to the instability of the endoperoxides and limited solubility of MUC we were unable to further characterize the complex reaction mixtures produced from singlet oxygenation. With this in mind we chose to study the reactions of MUC with MTAD, a singlet oxygen mimic. MTAD, a powerful electrophile, undergoes the same type of reactions as singlet oxygen, namely [4+2], [2+2], and the ene reactions.[20] We expected analogous products for the reactions of MTAD and singlet oxygen with MUC, but unlike the peroxide products formed from singlet oxygen, the expected [2+2] and [4+2] products from MTAD (Fig. 6) should be stable at room temperature and readily characterized using NMR.

t-MUC was dissolved in 0.5–1 mL of $CDCl_3$ in an NMR tube and 1 equiv of MTAD was added. Upon addition of MTAD to the *t*-MUC solution, the color immediately turns from red to deep purple, but quickly fades to a very light pink. Positive mode APCI-MS established the molecular ion, MH^+=266, indicating the addition of 1 equiv of MTAD. The proton NMR spectrum of the product shows a clean set of three triplets at 5.75, 5.49, and 5.24 ppm (1H each), with coupling constants ∼3.4 Hz, as well as singlets at 7.45 (1H), 3.05 (3H), and 3.75 ppm (3H). The spectrum does not contain peaks in the olefinic region with a characteristic trans coupling constant. The absence of trans olefinic peaks rules out the presence of **8** and **9**. The diazetidine product, **11** would exhibit a pair of doublets ∼5.0–6.0 ppm with characteristic

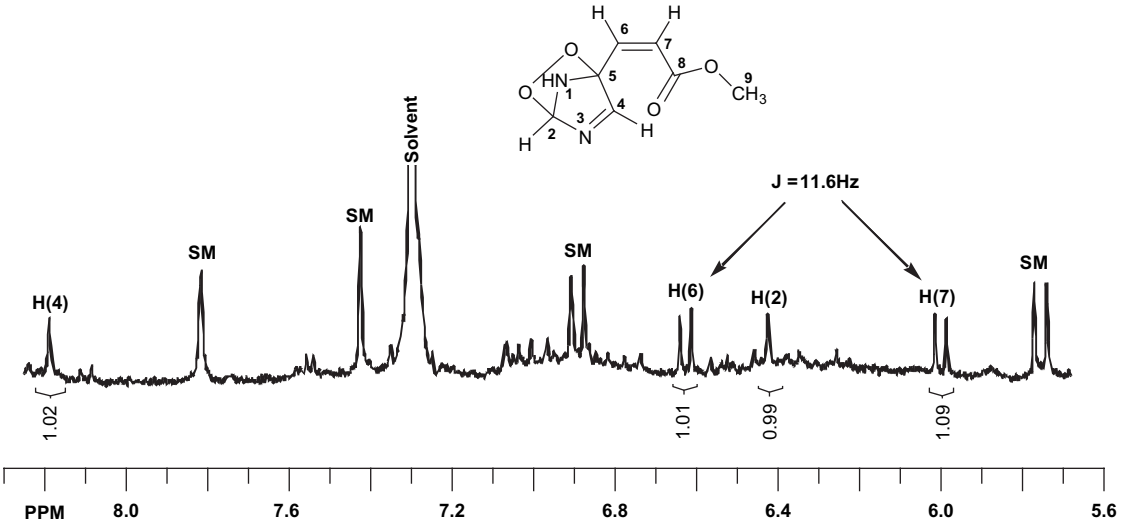

Figure 5. ^{1}H NMR spectrum of the products from reaction of singlet oxygen of *c*-MUC at 0 °C in $CDCl_3$.

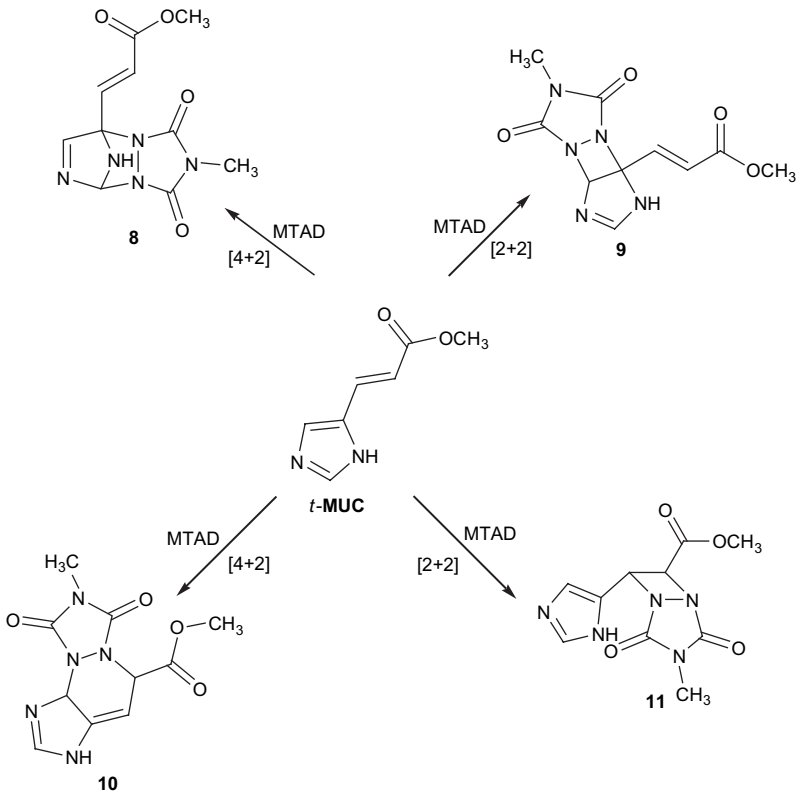

Figure 6. Likely products from the reactions of MTAD with *t*-MUC.

trans coupling constants[20c] as well as singlets corresponding to the imidazole ring hydrogens. The ^{1}H NMR spectrum does not possess peaks consistent with diazetidines. The [4+2] addition of MTAD across the olefinic side chain and the C_4–C_5 double bond of the imidazole ring yields **10**. While analogous [4+2] reactions of MTAD with styrenes have been reported,[21,22] the reaction product of MUC is not expected to lead to the observed set of triplets in the ^{1}H NMR spectrum. The H-shift product presented below (Fig. 7) would lead to the re-aromatization of the imidazole ring system possessing –CHH′–CH″– set protons and may exhibit three triplets in the ^{1}H NMR spectrum.

To test the presence of such a product, low temperature experiments were monitored by ^{1}H NMR at −30 °C. No rearrangement of the product was observed upon warming to 25 °C. The initial product, however, does slowly degrade at room temperature. Carbon, DEPT, and ^{1}H–^{13}C HETCOR NMR spectra were obtained at −30 °C. The DEPT spectra indicate that the reaction product does not contain any methylene groups ruling out the presence of the H-shifted product. Based on the NMR data summarized below (Figs. 8 and 9), we propose that the observed reaction product is **10**.

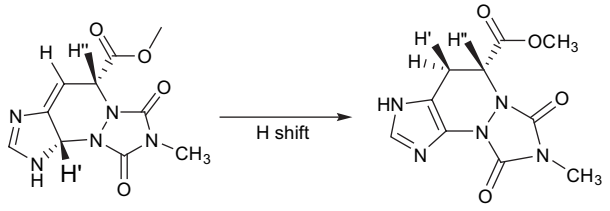

Figure 7. Potential H-shifted product of **10** *cis*.

The reaction of *c*-MUC with MTAD was run at −30 °C and exhibits the same color changes as the reaction of *t*-MUC. The proton NMR spectrum of the product contains three doublets of doublets at 5.93, 5.88, and 5.29 ppm (1H each) as shown in Figure 8. The spectrum indicates a single product and is consistent with, **10** *trans*, the [4+2] cycloaddition of MTAD across the double bond of the side chain and the C_4–C_5 double bond of the imidazole ring.

The ^{1}H NMR spectra of the MTAD products from the *cis*- and *trans*-MUC indicate each isomer leads to a single diastereomer (likely enantiotopic pairs). The observation of three clean triplets in the proton NMR spectrum of the *t*-MUC product and three doublets of doublets from *c*-MUC product is rationalized in terms of long range and different coupling constants due to geometrical differences. The results are in accordance with the Woodward–Hoffmann rules, with MTAD reacting in a disrotatory fashion to yield the cis or trans product depending on the stereochemistry of the starting material. One might expect **10** would be susceptible to a retro Diels–Alder transformation. However, upon heating to 50 °C they decompose yielding complex mixtures, which do not include MUC.

Addition of MTAD to a solution containing **10** *cis* results in its disappearance with the clean formation of a secondary product. The ^{1}H NMR spectrum of the secondary product has the following characteristics: a singlet at 7.93 ppm (1H), a pair of doublets at 6.01 and 5.37 ppm (1H each, J= 1.6 Hz), singlets at 3.80, 3.10, and 3.03 ppm (3H each). The ^{1}H and ^{13}C NMR spectra indicate a second equivalent of MTAD reacts via an ene reaction to form a stable 2:1 adduct. The reaction of MTAD with β,β-dimethyl-*p*-methoxy

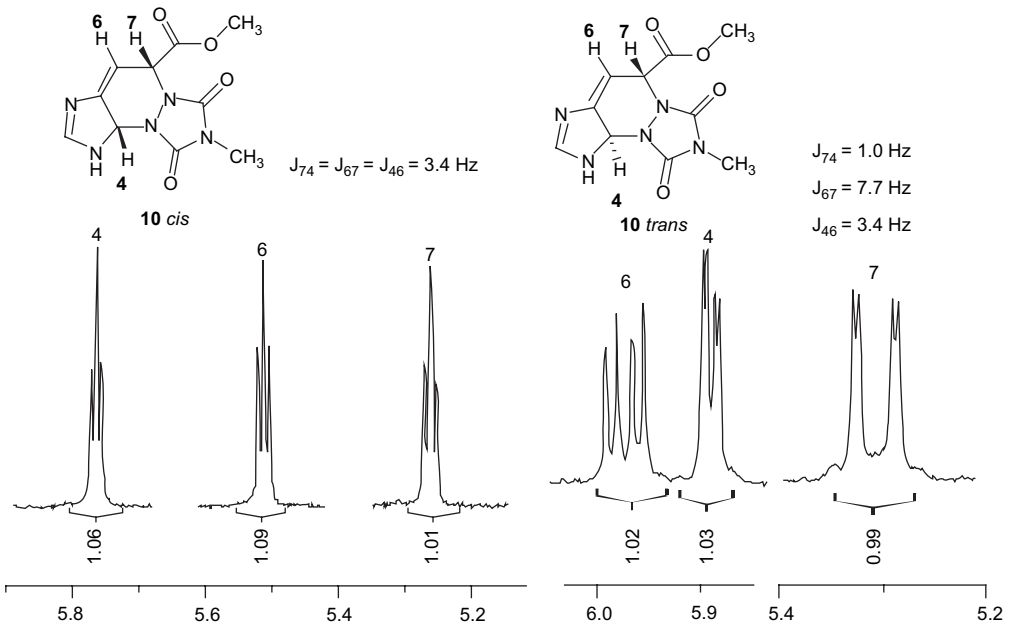

Figure 8. Expansion of ^{1}H NMR spectral regions for **10** *cis* and *trans*.

styrene yields an analogous 2:1 [4+2]/ene adduct, but via trappable dipolar intermediates.[21] Addition of MTAD to the **10** *trans* did not result in the formation of a 2:1 adduct at room temperature. While warming the solution to 50 °C promotes the formation of the 2:1 adduct as a minor product, the mixture readily decomposes.

Based on these results we propose the following mechanism (Fig. 10) for the reactions of MTAD with MUC. The addition of the first equivalent of MTAD to *trans*- and *cis*-MUC occurs via a suprafacial disrotatory [4+2] cycloaddition such that the relative stereochemistries of the homo-allylic protons are cis and trans, respectively. The cis homo-allylic protons in **10** *cis* are susceptible to the ene reaction with MTAD to form a 2:1 [4+2]/ene adduct, where as the ene reaction of MTAD with **10** *trans* is inhibited by steric factors induced by the trans geometry. It is also possible that the geometry of the allylic proton in **10** *trans* is not properly aligned (co-planar with the π system) for the ene reaction to occur.

To probe the involvement of dipolar intermediates in the reaction of MTAD with MUC,[23,24] 3 equiv of MeOH was added to the solvent prior to adding MTAD to MUC. Detailed analysis of the ^{1}H NMR spectrum from the MeOH trapping studies did not indicate the formation of any methanol adducts under our experimental conditions from either the cis or the trans isomers at −30 and 0 °C. While the formation of MeOH adducts is strong evidence for the involvement of dipolar intermediates the absence of such adducts suggest that dipolar intermediates are not involved or too short lived to be trapped.

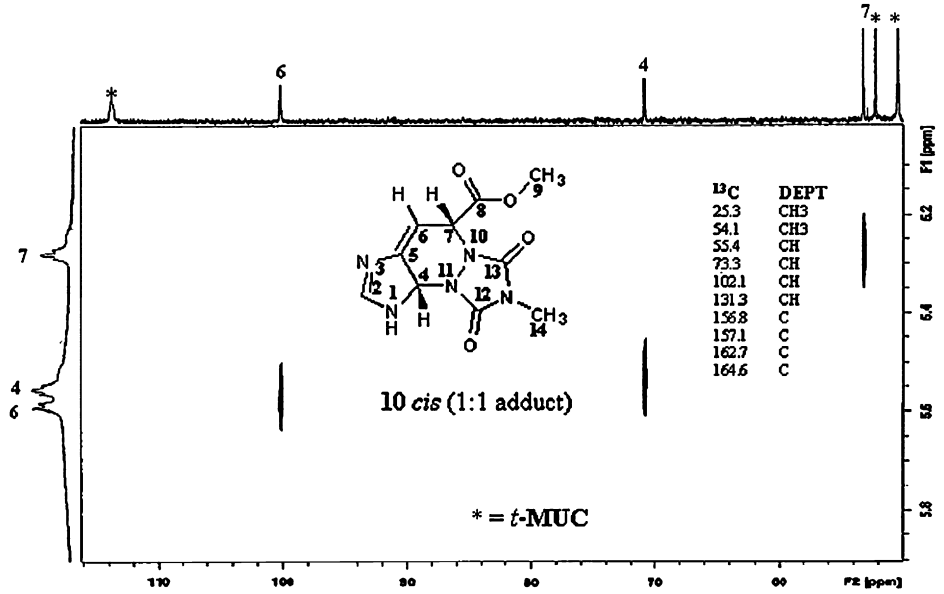

Figure 9. Summary of the ^{13}C, DEPT, and ^{1}H–^{13}C HETCOR NMR spectra for **10** *cis*.

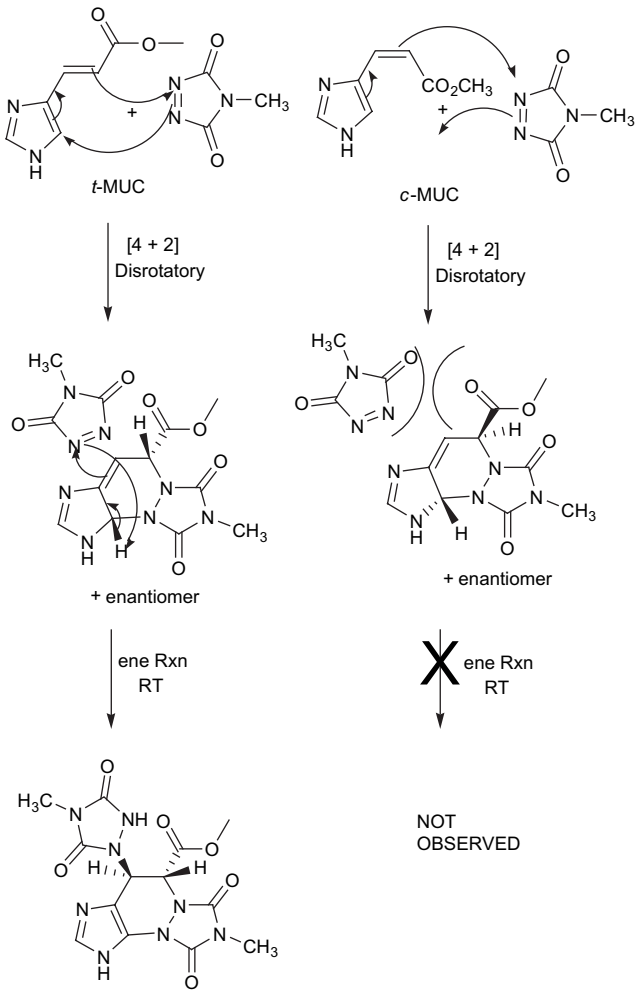

Figure 10. Proposed mechanism for the reaction of MTAD with *cis*- and *trans*-MUC.

Singlet oxygen and MTAD exhibit different reactivities toward MUC. One might expected that the imidazole ring system is more electron rich than the diene, which includes the enone side chain and C_4–C_5 double bond of the imidazole ring. If the reaction was controlled primarily by electronic factors, addition to the imidazole ring may be predominant as was observed with singlet oxygen. On the other hand, the observed [4+2] addition of MTAD involves the enone side chain. The observed different reactivities may be due to steric factors induced during secondary orbital interactions of MTAD with MUC (Fig. 11). The *endo* transition state leading to addition of MTAD to the imidazole ring will exhibit significant steric interactions between the *N*-methyl group on MTAD and the lone pair of the pyridine nitrogen atom of the imidazole ring. The [4+2] addition of MTAD involving the enone side chain, the observed reaction pathway, does not possess such steric interactions. Since singlet oxygen is not subject to such secondary orbital interactions or steric constraints a different reaction pathway is observed compared to MTAD.

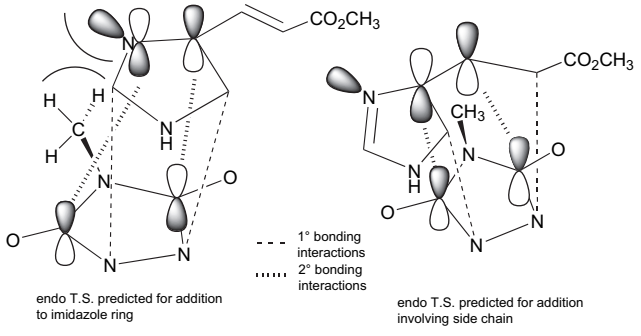

Figure 11. Possible transitions states for [4+2] additions of MTAD to *t*-MUC.

3. Conclusions

Singlet oxygen and MTAD exhibit dramatically different reactivities toward *cis*- and *trans*-MUC. Singlet oxygen undergoes [4+2] cycloaddition to the imidazole ring in MUC, while MTAD undergoes [4+2] stereospecific cycloaddition involving the olefinic side chain and C_4–C_5 double bond of the imidazole ring. The observed different reactivities may be due to secondary orbital interactions of MTAD during the cycloaddition and/or steric factors not presented by singlet oxygen. *t*-MUC forms a 1:2 MTAD [4+2]/ene adduct while the cis isomer only yields the [4+2] adduct at room temperature. While stereospecificity and absence of MeOH trapping adducts indicate the reactions of MTAD with MUC do not involve open or trappable dipolar intermediates, the vivid short lived color changes during the primary stages of the reactions indicate a charge transfer complex may be involved in the reaction process. The endoperoxides formed from the reaction of singlet oxygen with MUC are modestly stable at low temperature and decompose upon warming to form complex reaction mixtures. Studies are underway to characterize the products formed from the collapse of the MUC endoperoxides in an attempt to better understand the antioxidant role of UCA.

4. Experimental

4.1. General

Acetone-d_6, $CDCl_3$, CD_2Cl_2, CD_3CN, *t*-UCA, Rose bengal, cobalt(II) tetraphenylporphyrin, and MTAD were purchased from Aldrich and used as received. TLC analyses were performed on 0.2 mm thick plates pre-coated with fluorescent silica (Whatman), using short wavelength UV light to visualize the spots. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX-400 spectrometer equipped with a variable temperature probe. Chemical shifts values are in parts per million based on the chemical shift of the solvent. ^{13}C NMR peak multiplicity was determined by DEPT experiments. MS measurements were made using a direct probe on a Thermoquest Navigator Mass Spectrometer.

4.2. Reactions

4.2.1. Synthesis of *trans*-methyl, *n*-butyl, and *tert*-butyl urocanate. *trans*-UCA (1.0 g, 7.2 mmol) and 11 mL of 10% v/v solution of concentrated sulfuric acid in the appropriate alcohol were placed into a 50 mL round bottom flask. The solution was then heated to reflux for ~20 h. The resultant solution was allowed to cool to room temperature and 18 mL of the corresponding alcohol was added. The pH of

the solution was adjusted to 8–8.5 by adding solid anhydrous sodium carbonate. The basic solution was then gravity filtered and the solvent was removed under vacuum. The crude product was extracted with hot ethyl acetate. Evaporation of the solvent gave the desired ester in 90–95% yield. TLC analysis and column chromatography were performed using a 4:1 mixture of $CHCl_3$/EtOH. *trans*-Methyl urocanate: 400 MHz ^{1}H NMR (CD_3CN): δ (ppm) 7.63 (s, 1H), 7.57 (d, J=15.7 Hz, 1H), 7.33 (s, 1H), 6.41 (d, J=15.7 Hz, 1H), 3.69 (s, 3H); 100 MHz ^{13}C NMR: 167.3, 138.0, 136.4, 134.6, 123.8, 113.4, 51.3; *trans n*-butyl urocanate: 400 MHz ^{1}H NMR (acetone-d_6): δ (ppm) 7.65 (s, 1H), 7.53 (d, J=15.6 Hz, 1H), 7.30 (s, 1H), 6.39 (d, J=15.6 Hz, 1H), 4.12 (t, 2H), 1.55 (m, 2H), 1.30 (m, 2H), 0.92 (t, 3H); *trans tert*-butyl urocanate: 400 MHz ^{1}H NMR (acetone-d_6): δ (ppm) 7.64 (s, 1H), 7.55 (d, J=15.7 Hz, 1H), 7.32 (s, 1H), 6.40 (d, J=15.7 Hz, 1H), 1.38 (s, 9H).

4.2.2. Synthesis of *cis*-MUC. *trans*-MUC (0.760 g, 5 mmol) was mixed with 250 mL of dichloromethane and purged with argon for 2 min. The solution was prepared in a sealed quartz reaction vessel and irradiated in a Rayonet photochemical reactor equipped with sixteen 254 nm emitting bulbs for ~4 h at 34 °C. Analysis by ^{1}H NMR indicates a trans–cis ratio of 20:80. TLC analysis and column were performed using $CHCl_3$/EtOH in a ratio 9:1. ^{1}H NMR indicates the purified sample was of high purity with no traces of the trans isomer. 400 MHz ^{1}H NMR (CD_2Cl_2): δ (ppm) 7.70 (s, 1H), 7.34 (s, 1H), 6.88 (d, J=12.5 Hz, 1H), 5.70 (d, J=12.5 Hz, 1H), 3.78 (s, 3H); 100 MHz ^{13}C NMR: δ (ppm) 168.7, 137.4, 136.7, 131.2, 127.4, 110.7, 51.7.

4.2.3. Reactions of *cis*- and *trans*-MUC with singlet oxygen. MUC (2 mg, 13 μmol) was mixed with 1 mL of deuterated solvent (CD_2Cl_2, acetone-d_6, CD_3CN, or $CDCl_3$) in an NMR tube. Rose bengal (10 μM) was used as the sensitizer. A 200 W Hg–Xe lamp was used to irradiate the sample. A water filter was placed in front of the lamp as a heat filter and Pyrex glass was employed to filter light $\leq$320 nm. The NMR tube containing the sample was placed inside a windowed Dewar flask and oxygen was gently bubbled through the solution during irradiation. ^{1}H NMR spectra were taken at 5-min intervals at the same temperature of irradiation. The peaks in the ^{1}H NMR spectrum at low temperature assigned to endoperoxide, **1** *trans* are as follows: 400 MHz ($CDCl_3$): δ (ppm) 8.02 (s, 1H), 6.80 (d, J=16.1 Hz, 1H), 6.51 (d, J=16.1 Hz, 1H), 4.90 (s, 1H), 3.63 (s, 3H). The ^{1}H NMR signals for endoperoxide **1** *cis* are as follows: 400 MHz ($CDCl_3$): δ (ppm) 8.19 (s, 1H), 6.57 (d, J=11.6 Hz, 1H), 6.38 (s, 1H), 5.94 (d, J=11.6 Hz, 1H), 3.87 (s, 3H).

4.2.4. Reactions of *cis*- and *trans*-MUC with MTAD. MUC (2 mg, 13.1 μmol) was dissolved in 1 mL of deuterated chloroform. The solution was then reacted with 1 equiv of MTAD (1.5 mg, 13.1 μmol). The reactions of MTAD were carried out at temperatures down to −30 °C in a 5 mm NMR tube. NMR characterizations were run at the same temperature as the reaction. A summary of the chemical shift information for the major reaction products is provided below 400 MHz ($CDCl_3$): δ (ppm) 1:1 adduct, **10** *cis*; 7.45 (s, 1H), 5.75 (t, J=3.4 Hz, 1H), 5.49 (t, J=3.4 Hz, 1H), 5.24 (t, J=3.4 Hz, 1H), 3.75 (s, 3H), 3.05 (s, 3H); 1:1 adduct, **10** *trans*; 7.51 (s, 1H), 5.93 (d of d, J=7.7 and 3.4 Hz, 1H), 5.88 (d of d, J=3.4 and 1.1 Hz, 1H), 5.29 (d of d, J=7.7 and 1.1 Hz, 1H), 3.80 (s, 3H), 3.13 (s, 3H). 100 MHz (CD_3CN): δ (ppm) 25.3, 54.1, 55.4, 73.3, 102.1, 131.3, 156.8, 157.1, 162.7, 164.6. Positive mode APCI-MS of the **10** *cis* and *trans* yielded a molecular ion (MH^+: 266) indicating 1:1 adducts.

1:2 *t*-MUC/MTAD adduct 7.93 (s, 1H), 6.01 (d, J=1.6 Hz, 1H), 5.37 (d, J=1.6 Hz, 1H), 3.80 (s, 3H), 3.10 (s, 3H), 3.03 (s, 3H). 100 MHz (CD_3CN): δ (ppm) 24.8, 25.1, 50.1, 53.2, 60.0, 123.1, 132.4, 139.9, 153.5, 155.1, 155.4, 157.3, 168.4.

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