Master Dissertation Research Proposal

Synthesis of Urocanic Acid Derivatives and their Reactions with Singlet Oxygen

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INTRODUCTION

3-(1H-Imidazol-4(5)-yl)-2-propenoic acid commonly known as urocanic acid (UCA) is one of the primary UV absorbing chromophores present in the stratum corneum of human skin. UCA is formed naturally as the *trans* isomer (1,2). *t*-UCA isomerizes to *cis* form, upon absorption of UV light (Fig.1). This isomerization to the biologically active *cis* isomer is implicated in the photoinduced suppression of the immune system of skin. The initial rates of photoisomerization and photostationary *trans-cis* composition of urocanic acid correlate with solvent polarity with the exception of water (3). The *in vitro* photostationary state is approximately 30% *t*-UCA and 70% *c*-UCA under solar illumination (4). Because the urocanase enzyme is not present in skin, UCA accumulates until it is removed through sweat or during the monthly skin renewal process. UCA concentration within the human epidermis was estimated to be 0.3 to 8.9 mM based on several published studies (5).

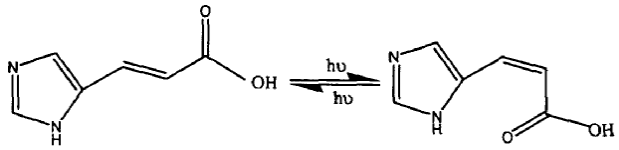


Fig1. Photoisomerization of *t*-UCA

c-UCA reportedly suppresses contact hypersensitivity and delayed hypersensitivity reduces the Langerhans cell count in the epidermis. *c*-UCA prolongs skin-graft survival time, and affects natural killer cell activity (6-8). The absorption coefficient of *c*-UCA is 70% of that of *t*-UCA at the absorption maximum 275 nm.

Due to its strong absorption of UV light UCA was considered to be a natural sunscreen and added to sunscreens and skin lotions to help prevent skin cancer and skin related diseases. However it was later discovered that photooxidation products of UCA can act as initiators of immunosuppression which may be critical to UV-induced pathogenesis of skin cancer and other cutaneous diseases (9, 10). This lead to considerable research into the photochemistry and photooxidative transformation of UCA. Recently de Fabo and co-workers reported evidence of *t*-UCA isomerization occurring throughout the UV-A region(11). Photochemical studies by Morrison and co-workers indicate radical production following the UV-A photoexcitation of *t*-UCA leads to DNA damage (12). A recent report clearly illustrates that photoexcited UCA can lead to the formation of singlet oxygen, and other reactive oxygen species (ROS). Reactive oxygen species are implicated in a number of diseases and disorders such as enzyme inactivation, mutations, premature aging, DNA damage and respiratory problems (13).

Peroxides are proposed photooxidation products of UCA and lead to damage of DNA. Singlet oxygenation of *cis-trans* methyl urocanate(MUC) yields 2, 5-endoperoxides which are stable at low temperature but decompose at room temperature to afford a complex product mixture.

SPECIFIC AIMS

Although mechanisms and products of UCA photooxidation are yet to be established, the photooxidation of UCA appears to be a significant factor in premature aging, skin cancer and UV induced immunosuppression. Despite the serious problems associated with the photooxidation products of UCA, the basic understanding of the reaction pathways is lacking and the photooxidation products have not been characterized.

1. Synthesis of *trans*-UCA esters: The reaction of singlet oxygen and UCA at room temperature gives a complex product mixture including unstable peroxides. In an attempt to characterize the primary singlet oxygen UCA reaction products, UCA ester derivatives will be synthesized with solubilities appropriate for low temperature NMR studies. Singlet oxygenation reactions will be carried out and characterized at temperatures down to -78°C.

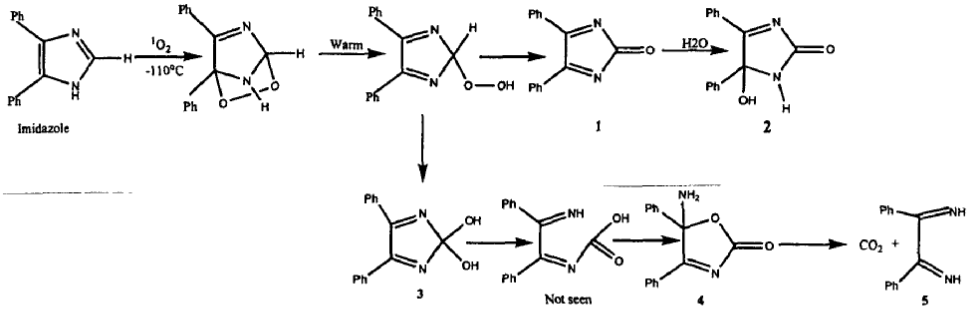
2. N-methylation of *trans* & *cis* UCA: The tautomeric nature of UCA leads to the presence of two sets of singlet oxygen products. To eliminate the tautomerisation, N-methyl UCA derivatives will be synthesized and their reactions with singlet oxygen studied.

3. Synthesis of labeled compounds: To characterize the UCA-singlet oxygen complex product mixtures we propose to synthesize ^{13}C and/or ^{15}N labeled compounds to enhance the applicability of heteronuclear NMR studies.

BACK GROUND

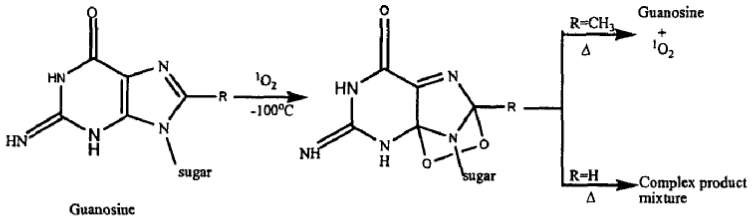
Extensive research has been conducted on the reactions of singlet oxygen with imidazole ring containing systems, especially histidine and guanine analogs. Early studies proposed the formation of 2, 5-endoperoxides in the reactions of singlet oxygen with substituted imidazoles depending on the substitution pattern of imidazole ring (14).The direct observation of imidazole 2, 5 endoperoxides as products of singlet oxygen were first reported by Ryang and Foote (15). Singlet oxygen reacts with 4, 5-diphenylimidazole via a [4+2] cycloaddition to form a 2, 5-endoperoxide which upon warming, decomposes to form a hydroperoxide. The hydroperoxide decomposes by two pathways; first via loss of water to form **1** which is hydrolyzed to **2**, second by decomposition to diols which rearrange to **4** then decomposes to CO_2 and benzyl diimine 5 as illustrated in scheme below (16). Kang and Foote conducted detailed product studies on isotopically labeled imidazole ring systems and found that reaction pathways are dramatically influenced by N-alkylation of parent compound (17).

Scheme 1: Reaction pathways of singlet oxygen with substituted imidazole.



Foote and coworkers suggested that singlet oxygenation of guanosine involves a series of reactive intermediates including endoperoxides, hydroperoxides and dioxeranes while these intermediates are stable at low temperatures upon warming to room temperature they decompose to form a complex mixture containing 20 products including spiroiminodihydantoin as the major product (18-20). The reaction pathways are highly sensitive to the substitution pattern only c-8 methylated substrate reacts to form an observable endoperoxides, which decomposes to yield singlet oxygen and starting substrate.

Scheme 2: Reactions of singlet oxygen with guanosine



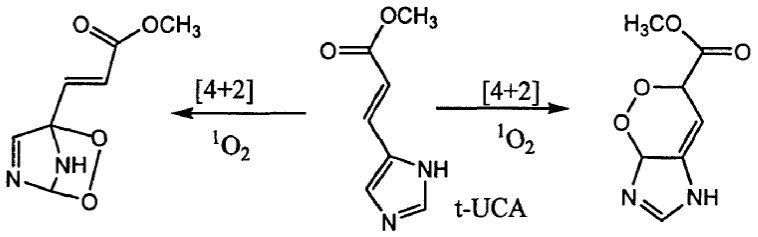
RECENT AND PROPOSED STUDIES:

UCA can generate singlet oxygen upon absorption of UV light. Singlet oxygen then converts UCA to products that catalyze the further destruction of UCA forming a yellow solution after extended irradiation. The Z isomer is 1.4 times more reactive towards singlet oxygen than the E isomer is (21). The photodegradation of UCA by its photoproducts is oxygen and singlet oxygen dependent. The product mixture generates higher levels of singlet oxygen, UVA light, possibly because of greater light absorption at these wavelengths. The photoproducts include peroxides which absorb UVA leading to free radicals.

$$\text{t-UCA} \xrightarrow{UV} \text{t-UCA}^* \longrightarrow \text{t-UCA}^3 \xrightarrow{^3O_2} {}^1O_2\text{UCA} \longrightarrow \text{UCA}_{[ox]} \xrightarrow{\text{UCA}} 2\text{UCA}_{[ox]}$$

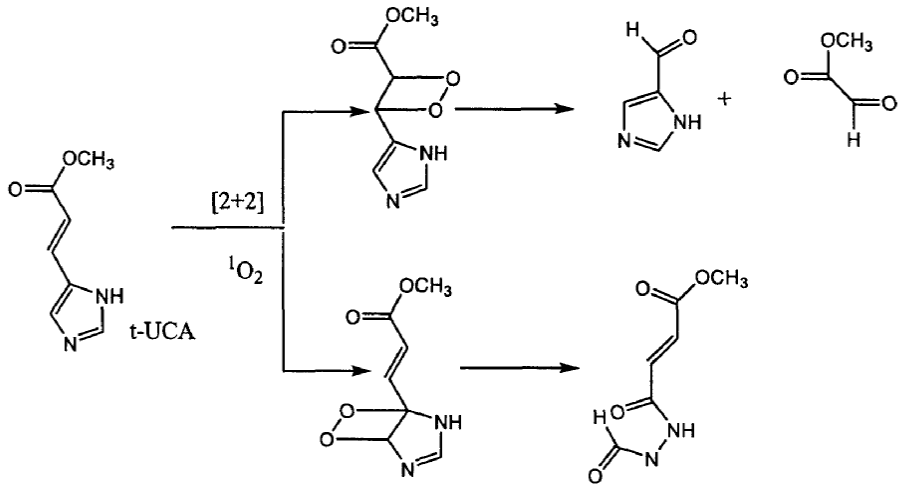
Because of insoluble nature of UCA in organic solvents derivatives will be used as a model. Simple ester analogs will be synthesized by acid promoted esterification. The methyl ester(MUC) is modestly soluble in organic solvents required for low temperature NMR studies. MUC is expected to exhibit the same reactions as the UCA towards singlet oxygen. The expected reaction pathways are discussed in the following section. Singlet oxygen is a strong electrophile and often undergoes "4+2" (Diels-Alder) type cycloaddition with imidazole to form 2, 5-endoperoxide 1. In the case of UCA, singlet oxygen may also undergo "4+2" addition across the double bond of the side chain and the C4-C5 double bond of the imidazole ring to yield endoperoxide 2,as shown below(22).

Scheme 3: [4+2] additions of singlet oxygen with MUC



Singlet oxygen can also react via "2+2" cycloaddition to form dioxeranes which typically decompose to the corresponding compounds. The addition of singlet oxygen via "2+2" can occur at the C4-C5 double bond of the imidazole ring or at the side chain to form the dioxetanes. The dioxetanes are expected to collapse to the corresponding carbonyl compounds as shown below.

Scheme 4: [2+2] additions of singlet oxygen with MUC

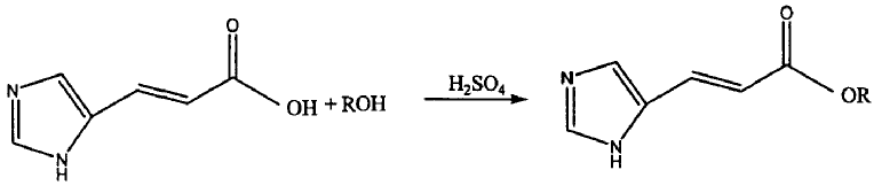


H-NMR and GC-MS analysis from our research group show no evidence of singlet oxygen reactions at the side chain of t-MUC. The room temperature ^{1}H-NMR spectrum of UCA oxidation products gives an almost continuous series of overlapping peaks between 6 and 8 ppm indicating complex mixture of products at room temperature. The proton spectrum of the reaction mixture at low temperature indicate at least two endoperoxides are formed which readily decompose to the complex product mixture upon warming to room temperature.

EXPERIMENTAL SECTION

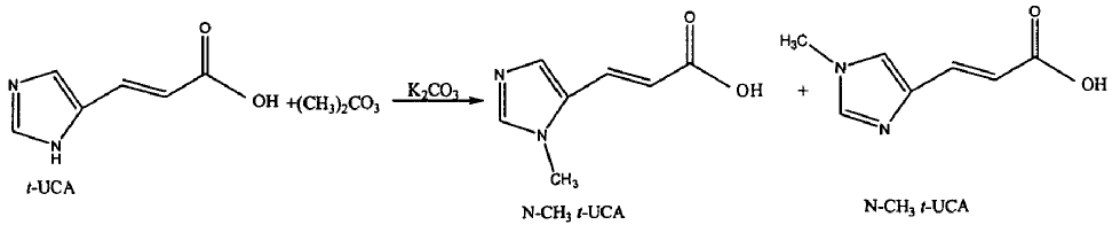
1. Synthesis of *cis* & *trans* UCA esters: *trans* UCA esters can be prepared by acid catalyzed esterification using various alcohols (23). *trans* UCA can be isomerized to *cis* UCA by photolysis. The *trans* and *cis* isomers can be separated by chromatography (4).

Scheme 5: Esterification of *t*-UCA



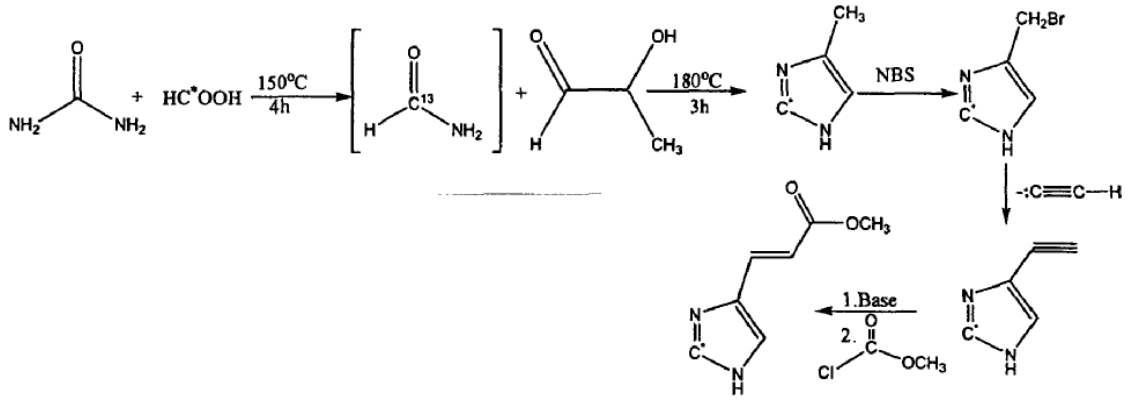
2. N-methylation of t-UCA: N-methyl *trans* UCA can be prepared by using dimethyl carbonate and potassium carbonate. Alternatively diazomethane will be used in an attempt to methylate UCA (24).

Scheme 6: N-methylation of *t*-UCA



3. Synthesis of labeled compound: Proposed synthesis for ^{13}C label UCA will be attempted following literature methods for synthesis of labeled imidazole (17)

Scheme 7: Proposed synthesis of ^{13}C labeled UCA



Multi Labeled urocanic acid was synthesized by the enzymatic reaction of DL-[3,3-$^{2}H_2$,1',3'-$^{15}N_2$] histidine or DL-[2,3,3,5'-$^{2}H_4$,2'-^{13}C,1',3'-$^{15}N_2$] histidine with histidine ammonia-lyase at pH9.0 (25).

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