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Urocanic Acid Photobiology. Purine-Assisted Photooxidation to 1H-Imidazole-4(5)-Carboxaldehyde.[1]

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Abstract: *Urocanic acid undergoes photooxidative cleavage at the acrylic acid side chain to afford imidazole carboxaldehyde. The reaction is accelerated in the presence of purines. Evidence is presented that the photooxidation involves a reaction of the UA radical cation and ground state molecular oxygen.*

Urocanic acid (3-(1H-imidazol-4(5)-yl)-2-propenoic acid, UA), a metabolite of histidine that accumulates in the mammalian epidermis, is of considerable current photobiological interest.[2] The naturally occurring *trans* i.e. (E) isomer is efficiently converted by sunlight to *cis* i.e. (Z)-UA[3], a compound implicated in the phenomenon of immunosuppression.[4] UA also photochemically covalently binds to protein[5] and DNA[6], and photoinactivates infectious viral ss- and dsDNA.[2,7] There is good evidence that thymidine is a major target base in DNA[8] and [2+2] cycloadducts involving the 5,6-double bond of thymidine and the olefinic moiety of UA have been isolated and identified.[9] However, we have reported that photolysis of radiolabeled UA with poly[A] leads to substantial incorporation of the label into that nucleic acid as well.[8] The chemical basis for the binding of UA to the deoxyadenosine unit is as yet unclear and we have studied the chemistry resulting from the photolysis of UA in presence of dA as part of our effort to understand how UA and dA interact. We report herein a novel photooxidative cleavage of the acrylic acid double bond which is facilitated by dA and by dG.

Irradiation of an aqueous solution of UA (2.0 mM) and dA (14.0 mM), in the presence of oxygen, with a medium-pressure 450W Hanovia mercury arc lamp encased in a corex sleeve (transmitting wavelengths greater than 270 nm) produces 1H-imidazole-4(5)-carboxaldehyde (ImCHO) (equation 1). This is the sole photoproduct of UA detectable when the photolysate is analysed by reversed-phase HPLC monitoring the column effluent at 254

(E)-UA   +   dA   or   dG $\xrightarrow{h\nu/H_2O/O_2}$ ImCHO   (1)
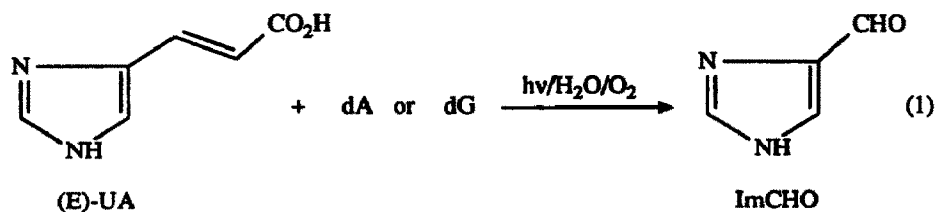
nm. The identity of the aldehyde, collected by HPLC from a scaled-up photolysis, was confirmed by spectroscopic analysis[10] including comparison with an authentic sample prepared by manganese dioxide oxidation of 4(5)-hydroxymethyl-1H-imidazole.[11] The aldehyde is formed in the absence of dA, but only at ca. 20% rate with the purine present. The presence of dG also catalyzes the reaction at a level commensurate with that observed for dA (see below). Experiments with UA and dA in phosphate buffer of varying pH (from 5 to 8) showed no significant difference in the yield of aldehyde formation.

The source of the oxygen incorporated into the aldehyde was probed by irradiating UA in methanol in the presence of $^{18}O_2$ (NaN$_3$ was added to increase the yield of aldehyde; see below). A CI-high resolution mass spectrum of the isolated aldehyde gave m/z 99.0443 ($C_4H_4N_2^{18}O$ = 99.0444) with 75% incorporation of oxygen-18 determined by using the molecular ion (M+H$^+$) of the ImCH^{18}O at 99 m/z (CIMS). Though the formation of ImCHO can be envisaged as arising from the addition of singlet oxygen ($^1O_2$) to give an initial dioxetane, several facts do not support this hypothesis: i) singlet oxygen is known to react efficiently with the imidazole ring in histidine,[12] ii) independent generation of $^1O_2$ with rose bengal gave no evidence for the formation of ImCHO from UA, (though UA was degraded), iii) D$_2$O did not enhance the yield of the aldehyde nor did the addition of sodium azide retard the reaction; these reagents are now classic probes for singlet oxygen chemistry.[13,14]

Interestingly, the presence of sodium azide was found to actually enhance the yield of ImCHO. The presence of the salt also improved the mass balance (i.e. after 4 h aldehyde accounted for 36% and 16% of degraded UA in the presence and absence of azide, respectively).[15] Catalysis by dG is also facilitated by the presence of the azide, with the ImCHO accounting for as much as 55% of reacted UA under these conditions. Quantum efficiencies (more accurately "quantum utilizations" because multiple absorbers are present) for the formation of ImCHO are presented in Table 1.

**Table 1. "Quantum Utilization" for the Formation of ImCHO in the Photolysis[a,b] of UA and Purines.[c]**

Reactants	± NaN$_3$	Φ x 10^4
UA/dA	-	0.71 (± 0.7%)
UA/dA	+	1.39 (± 2.5%)
UA/dG	-	0.85 (± 4.1%)
UA/dG	+	2.04 (± 1.9%)

[a] Using 266 nm light from a Continuum Nd:YAG laser, pulse energy 4.7 mJ. [b] Comparable results were obtained from the photolysis of UA/dA in the absence and presence of NaN$_3$ with the 308-nm light from a Lumonics EX-700 Pulsemaster XeCl excimer laser, pulse energy 14.6 mJ. [c] 2.0 mM UA and 14.0 mM purines. Purines are the major absorbers of light at these wavelengths. All data are the results of duplicate measurements.

Since singlet oxygen is not involved, we are left with two possibilities for the oxygen source, i.e. the superoxide radical anion (O$_2^-$) or ground state molecular oxygen ($^3O_2$). In fact, we have elsewhere shown that UA generates superoxide when irradiated at wavelengths > 270 nm.[16] Involvement of superoxide was tested by irradiating UA and UA/dA in the absence and presence of superoxide dismutase (SOD). The enzyme had only a minor effect on the rate of the oxidation.[17] We thus propose that ground state oxygen is involved and that the cleavage of UA involves single electron transfer (SET) processes.

The key species is the UA radical cation, formed either by direct photoionization[16] or by electron transfer from UA to initially generated purine radical cations. Strong evidence in support of a reaction involving the UA radical cation and $^3O_2$ was provided by independently generating the UA radical cation by the oxidation of UA through visible light excitation of the highly electron affinic dye, nitro blue tetrazolium (NBT).[16] HPLC analysis confirmed that ImCHO is indeed formed under these conditions and, when NaN_3 was present, in yields as high as 68%. It is noteworthy that NBT is a known quencher of O_2^{-}.[16] Furthermore, there is ample precedent for oxidative cleavage of photogenerated olefin radical cations.[19] Azide catalysis could derive from its ability to trap the UA radical cation and inhibit competitive hydrolysis. There is good precedent for azide[14] functioning in this role.[20] A complete mechanism is shown in Scheme 1 and includes both the chemistry resulting from direct excitation of UA as well as that arising from initial purine excitation.[21]

Scheme 1
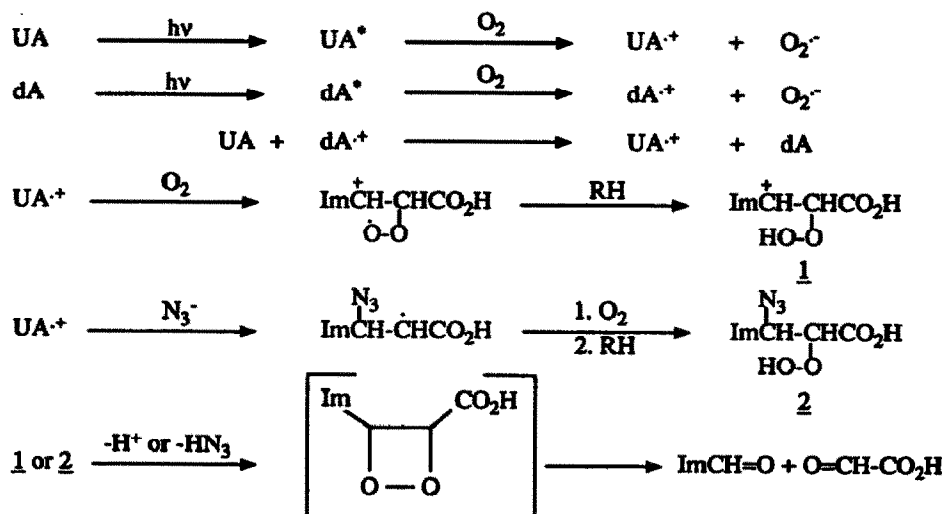
A proposal somewhat analogous to the role of dA in Scheme 1 has recently appeared to explain the dimethoxybenzene (DMB) sensitized photooxidation of alkenes to ketones and aldehydes.[22] Here it is proposed that the DMB photoionizes and the $DMB^{.+}$ converts the alkenes to their radical cations. Superoxide has been invoked as the reagent which leads to (undetected) dioxetanes, but in this and related cases the involvement of ground state oxygen is more likely. [20,23]

The generality of this purine-assisted photooxidative cleavage of UA, and its potential role in the photolytic binding of UA to nucleic acids, are currently under study.

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10. UV (H_2O):λ_{max}, 257 nm; ^1H NMR (D_2O): δ 7.90 (s, 1H, C4(5)-H), 8.02 (s, 1H, C2-H), 9.66 ppm (s,1H, CHO); ^{13}C NMR (D_2O): δ 130.8, 138.5, 139.6, 186.3 ppm; EIMS: m/z (relative intensity) 96 ($M^{+\cdot}$, 100), 68 (30), 67 (77); CIMS: m/z (relative intensity) 97 ($M+H^+$, 100).

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21. For simplicity UA is drawn as the non-ionized species; depending on the pH of the medium it can also exist in an ionized form. The photoisomerization is quite fast under these conditions and the rate of aldehyde formation from (E)-UA and (Z)-UA is indistinguishable in the presence of NBT. Moreover, the two isomers do not differ significantly in their photoionization efficiencies (C. Lifshitz, private communication).

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