Photooxidation of Acyclovir with Thermally Generated Triplet Excited Ketones. A Comparison with Type I and II Photosensitizers

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The antiviral drug acyclovir (Ac, 1) was treated with triplet excited ketones, which have been generated in thermal decomposition of 3-(hydroxymethyl)-3,4,4-trimethyl-1,2-dioxetane (HTMD), in the dark. Three major oxidation products were detected by means of spectroscopic measurements. The products were (2-hydroxyethoxy) methyl spiroiminodihydantoin (2), (2-hydroxyethoxy) methyl (amino)-2-imino-1,2-dihydroimidazole-5-one (3), and 2,2-diamino-4-[(2-hydroxyethoxy) methyl) amino)-5-[2*H*]-oxazolone (4). Equal amounts of type I and type II photooxidation products were found, as could be established by comparison with predominant type I (riboflavin) and type II (rose bengal) photosensitizers. The concentration and time profiles for the HTMD-induced oxidation of Ac were also determined. The participation of singlet oxygen in HTMD-induced oxidation was confirmed by the substantial D_2O effect in the formation of spiroiminodihydantoin (2).

Key words acyclovir; photooxidation; 1,2-dioxetane; triplet-excited ketone

Over the last decade there has been increasing interest in the photosensitization mechanism in the biological systems, in relation to both deleterious and therapeutic aspects of this phenomenon. Photosensitization reactions are generally considered as belonging to either the type I (radical mediated) or type II (singlet oxygen mediated).[1)] Many of the endogenous (*e.g.* flavins, tetrapyrrols, protoporphyrins *etc.*) or exogenous photosensitizers can elicit phototoxic or photoallergic responses.[2,3)] Likewise there are many sensitizing dyes *e.g.* rose bengal, benzophenone, riboflavin *etc.* that are activated by light.

An important class of photooxidative sensitizers constitutes the triplet-excited ketones,[4,5)] which are of biological interest since they may be generated in cellular systems upon exposure of endogenous chromophores to the UV irradiation or by dark reactions (*e.g.* lipid peroxidation and enzymatic oxidation).[6—8)] Triplet excited ketones may be produced by thermal decomposition of 1,2-dioxetanes, alternative to their conventional photochemical generations.[9,10)] These thermally generated triplet-excited ketones are analogous to those produced photochemically and operate as type I or type II photooxidants.[11)] 1,2-Dioxetanes are unique class of four membered ring peroxides and are of biological interest, since they have been implicated as labile intermediates in oxidative stress.[5)]

Acyclovir (9-[(2-hydroxyrthoxy) methyl) guanine) (**Ac**, **1**) is an antiviral drug used for the treatment of herpes encephalitis caused by herpes simplex virus or varicella zoster virus infections. In our recent study for the singlet oxygen mediated photooxidation of **Ac** in aqueous solution,[12)] we have isolated spiroiminodihydantoin (**Sp**, **2**), imidazolone (**Im**, **3**) and oxazolone (**Ox**, **4**) as the three major products (Chart 1). Herein we have investigated photooxidation of **Ac** in presence of thermally generated triplet excited ketones. The distribution of products were compared with those of **Ac** photooxidation by a type I sensitizer, riboflavin and a type II sensitizer rose bengal.[13,14)]

Experimental

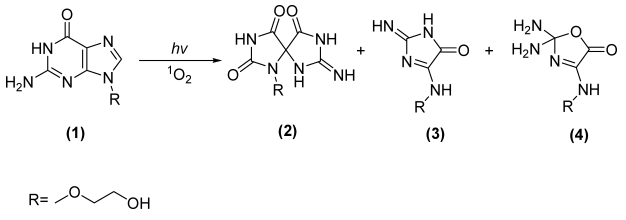
Chemicals and Reagents All chemicals used were of analytical grade. Acyclovir was extracted from the commercial medicament Acivir (Cipla Limited, Mumbai, India) with a soxhlet extractor, purified by TLC and recrystallized from the same solvent. Melting point, ^{1}H-NMR and co-TLC with the authentic pure sample determined the purity of acyclovir. HTMD was synthesized according to the literature procedure.[15)] Standard samples of Spiroiminodyhydantoin, imidazolone and oxazolone were prepared by rose bengal photosensitized oxidation of **Ac**.

Oxidation Procedure For the dioxetane mediated oxidation of **Ac**, a 0.5 mM solution of **Ac** in 10 mM sodium cacodylate buffer (pH 7.0) and 10 vol% dioxetane solution in acetonitrile was kept at 50 °C for several hours in absence of light. The photosensitized oxidation of phosphate buffered solution of **Ac** (0.5 mM) was carried out in the presence of photosensitizers riboflavin or rose bengal. A 150 W sodium lamp was used for the carrying out photosensitized oxidations of **Ac** with riboflavin and rose bengal. The lamp was placed at 15 cm below the bottom of an ice filled beaker, in which a round bottom flask having reaction mixture was placed. HTMD-induced photooxidation was carried out in dark for 18 h at 50 °C by using acetonitrile (10% by vol) as co-solvent while the photooxidation by riboflavin and rose bengal was carried out for 2.5 h. A number of products were indicated on TLC at the end of the reaction, from which only three major photoproducts **2**, **3** and **4** were obtained in isolable yields. After the given time period the amount of photoproducts formed and un-oxidized **Ac** was assessed by isolation and purification of the photolysate using silica gel column chromatography.

Relative yields of products were determined based on the consumed **Ac** and the mean value of at least 3 independent runs was reported (Table 1). The concentration and time profile for HTMD induced oxidation of **Ac** (0.5 mM) at 50 °C were determined by using 10 mM sodium cacodylate buffer (pH 7) as a reaction medium with 10 vol% acetonitrile as co-solvent. Effects of D_2O on the yield of **Sp** in HTMD-induced photooxidation were also observed to confirm the involvement of singlet oxygen.

Results and Discussion

On the thermal treatment of **Ac** with **HTMD** at 50 °C three major products were identified. The products were (2-hydroxyethoxy) methyl spiroiminodihydantoin (**2**), (2-hydroxyethoxy) methyl (amino)-2-imino-1,2-diydroimidazole-



(1) (2) (3) (4)

Chart 1

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5-one (**3**), and 2,2-diamino-4-[(2-hydroxyethoxy) methyl)-amino)-5-[2*H*]-oxazolone (**4**) (Chart 2). All the photoproducts were identified by comparing their spectral data and TLC with those of authentic pure samples of spiroiminodihydantoin (**Sp**), imidazolone (**Im**) and oxazolone (**Ox**) generated in the rose bengal mediated photooxidation of **Ac**. The spectral data of all the three products were found to be in close agreement with the well-established singlet oxygen mediated **Ac** photooxidation products[12] (Chart 1). Similar products pattern was obtained when **Ac** was exposed in aerated aqueous solution of photoexcited riboflavin and rose bengal. Different yields of products were obtained depending upon the photosensitizer, which was used. In the type II sensitized photooxidation by rose bengal, spiroiminodihydantoin was detected as the major product whereas the type I photosensitizer riboflavin gave imidazolone and oxazolone as major product. For HTMD, which entails both photooxidation modes, the yields of type I and type II products were in close agreement. The **Sp**/(**Im**+**Ox**) product ratio may serve as mechanistic probe to access the predominant photooxidation products in type I and type II photosensitized oxidative modifications. For example in riboflavin sensitized photooxidation the product ratio lies below 1 while for rose bengal sensitized oxidation it is above 3. For HTMD the product ratio is 1.3. Also for the product balance, the HTMD oxidation lies between the type I (40%, entries 2) and type II (57%, entries 3) process. Furthermore the relative yield of **Sp** in HTMD-induced oxidation (30%) is significantly higher (15%) than that of riboflavin sensitized oxidation and smaller (15%) than that in the rose bengal sensitization. In contrast the yields of the type I products in HTMD-induced oxidation (23%) are comparable with those of type I sensitizer (25%) and significantly higher than those in type II photooxidation (12%).

Table 1. Product Balance of **Ac** Oxidation by Dioxetane HTMD and by Riboflavin and rose bengal Photosensitizers

Entry	Concentration (μM)	Oxidant	Product yields (%)[a]				Product balance
			Type II	Type I			
			Sp	**Im**	**Ox**	Total	
1	10000	HTMD/50 °C	30±3	8±2	15±1	23±2	53±3
2	10	Riboflavin	15±2	7±1	18±2	25±3	40±3
3	10	Rose bengal	45±1	5±1	7±1	12±2	57±2

a) Relative yields based on consumed **Ac**, mean value of three independent runs.

This difference in products distribution may be accounted for the fact that rose bengal produces singlet oxygen in large amount by a type II mechanism whereas riboflavin, which act mainly by type I photosensitized oxidation do not produce significant amount of singlet oxygen. On the other hand HTMD, is neither a typical type I nor a characteristic type II photooxidant; in particular both photooxidation modes occur quite efficiently.

As shown in Fig. 1, when **Ac** was thermally treated with HTMD at 50 °C for 15 h, a linearly dependent degradation of **Ac** was observed with increasing HTMD concentration. The absolute yield, based on initial amount of **Ac**, of characteristic type I photooxidation products, **Ox** and **Im** was 15%, while type II product **Sp** was formed in up to 22% absolute yield at 25 mM HTMD concentration. In these reactions the sum of quantified products, relative to consumed **Ac**, amounted to be 56±4% and was independent of the HTMD concentration. Figure 2 shows the time profile for thermally HTMD induced photooxidation of **Ac**, which revealed a gradual increase of all oxidation products with time and product balance of 53±3% at 25 mM HTMD. Also in this case the product balance was independent of the reaction time.

The linear increase of **Ac** conversion with increasing HTMD concentration suggests that the HTMD oxidation of **Ac** is directly proportional to the amount of triplet-excited ketones formed by the thermal decomposition of the dioxetane. The time profile for photooxidation of **Ac** exhibited a decrease of **Ac** concentration with time. This reflects that the **Ac** is consumed in parallel with the generation of triplet-excited ketones.

Singlet oxygen quenchers such as DABCO, sodium azide *etc.* cannot be used to confirm the involvement of singlet oxygen in HTMD mediated photooxidation because they react with dioxetane.[16,17] Proof for the involvement of singlet oxygen in HTMD-induced oxidation comes from the substantial effect of D_2O on the formation of **Sp**. Higher yields of **Sp** in D_2O compared to those of H_2O implicate that singlet

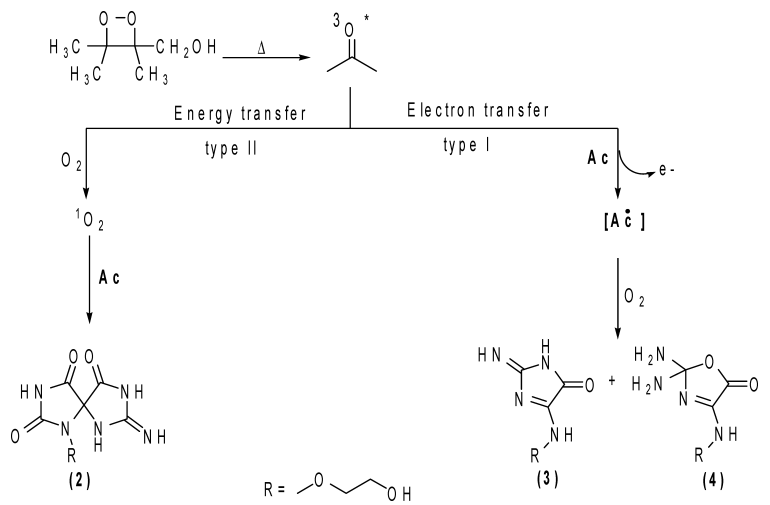

Chart 2

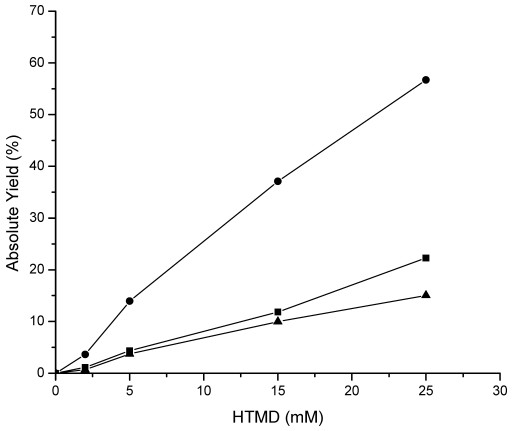

Fig. 1. Concentration Profile for the Thermally HTMD-Induced Photooxidation of **Ac**, Yields Derived from the Mean Values of Three Independent Runs, (●) Conversion **Ac**, (▲) Yields of **Ox** and **Im**, (■) Yields of **Sp**

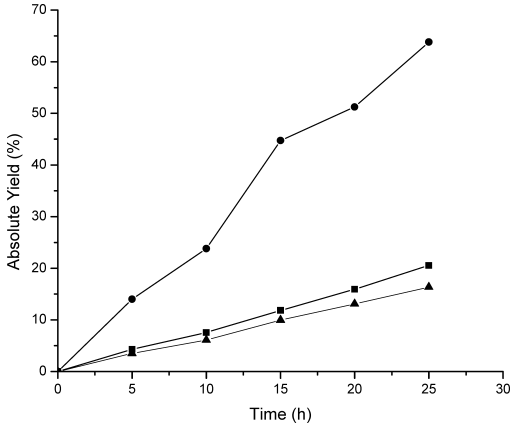

Fig. 2. Time Profile for the Thermally HTMD-Induced Photooxidation of **Ac**, Yields Derived from the Mean Values of Three Independent Runs, (●) Conversion **Ac**, (▲) Yields of **Ox** and **Im**, (■) Yields of **Sp**

oxygen is involved in HTMD mediated photooxidation.

Our present investigation reveals that triplet excited ketones generated in the thermal decomposition of HTMD oxidize **Ac** efficiently to the **Sp**, by a type II photooxidation mechanism and to the **Ox** and **Im** by a type I mechanism. In addition, in the riboflavin sensitized type I oxidative modification of Acyclovir **Ox** and **Im** was obtained as major product whereas **Sp** was characterized as a major type II photooxidation product of rose bengal sensitized oxidation of **Ac**.

The investigation of photochemical properties of compounds used in clinical medicines is of great relevance from photobiological as well as photomedical point of view. Since singlet oxygen formation and the ensuing photooxidation of the drug and biomolecules is one of the main routes for the drug phototoxicity, the present findings may have an implication to the phototoxic effect of the drug.

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