Chapter 2

Photochemical Studies on Acyclovir and

Phenazopyridine Hydrochloride
**Introduction**

The treatment of disease requires the use of either systemic or topical medication during certain period of time. Frequently the treatment coincides with exposure to electromagnetic radiations, coming from different types of sources (sunlight in works made outdoor and intense artificial radiation used in specific works etc.). This coincidence may lead to photobiological effects such as drug photosensitization, phototoxicity, photodegradation etc. The molecular mechanism of biological photosensitization induced by drugs and their phototoxicity is receiving increasing attention.\(^1-5\) With regard to the mechanistic pathways, it is accepted basically the four paths as main routes for phototoxic reactions,\(^5\) namely *singlet oxygen formation and its reaction with drug, radical formation, covalent photobinding to biomolecules and photoproducts in decomposition reaction.*

Studies on both, the drug induced singlet oxygen formation and its reaction with drug and photodegradation of drugs are relevant to drug development process, because the photoproducts may have biological effects different from those of parent compounds. These studies are of high significance in current medicinal chemistry as this may explain, at least partially, phototoxicity mechanism.

With this interest herein we have investigated:

[A] 2. Photooxidation of acyclovir by thermally generated triplet excited ketone from 1,2-dioxetane and its comparison with type I and type II photosensitizers.

[B] Photochemistry of Phenazopyridine hydrochloride.
Section [A]

*Photooxidation Studies on Acyclovir*
[A]. 1. Photooxidation of acyclovir in aqueous solution

Acyclovir (9-[(2-hydroxyethoxy) methyl] guanine) (Ac, 1) is an antiviral drug used for the treatment of herpes encephalitis caused by herpes simplex virus or varicella zoster infections. Acyclovir is mainly used to treat chickenpox, shingles, and the symptoms of herpes virus infections of the genitals, lips, mouth, skin, and brain. The medicine does not cure the infections, but it relieves the discomfort and speeds healing of sores, when they are present.⁶

Although acyclovir has a large therapeutic index and usually well-tolerated, acute renal failure and neurotoxicity are two important potential adverse effect of this drug.⁷ ⁸ It is metabolized, probably by alcohol dehydrogenase and aldehyde dehydrogenase, to 9-carboxymethoxymethyl guanine and to a smaller extent to 8-hydroxy-9-(2-hydroxyethoxymethyl) guanine (8-OH-Acyclovir).⁹ Acyclovir is structurally related to deoxyguanosine and with photoactivatable chromophores and electron rich heterocyclic ring it is prone to photochemical transformation, including reaction with the electrophilic singlet oxygen.

Experimental

Chemicals

All chemicals used were of analytical grade. Acyclovir was extracted, from the commercial medicament Acivir (Cipla Limited, Mumbai, India) with a soxhlet extractor using methanol as the solvent and recrystallized from the same
solvent. Melting point, $^1$H-NMR and co-TLC with the authentic pure sample determined the purity of acyclovir.

**Apparatus**

Irradiations were carried out in a photoreactor equipped with medium pressure mercury vapour lamp (Philips, 450 W) inserted in a water-cooled immersion well with continuous supply of water. The incident photon flux of the irradiation setup was $9.78 \times 10^9$ einstein/min as determined by using ferrioxalate actinometry. IR spectra were recorded as KBr discs on a Perkin Elmer model spectrum RX1. $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Bruker DRX-300 spectrometer using SiMe$_4$ as internal standard. EIMS and FAB-mass spectra were recorded on VG-ZAB-HS and Jeol SX 102/DA-6000 mass spectrometers at 10 KV accelerating voltage spectrometer using m-nitrobenzyl alcohol (NBA) matrix and argon as FAB gas, respectively. High resolution mass spectra were determined with a VG-ZAB-BEQ9 spectrometer at 70 eV ionization voltage.

**Photooxidation procedure**

Irradiation of an aqueous solution of Ac (5 mM) in 50 mM phosphate buffer (pH 7) was carried out in, the absence and in presence of rose bengal (0.1mM) as sensitizer, with medium pressure mercury vapour lamp. The solution was continuously stirred during photolysis and the temperature of the solution was kept at 15°C during irradiation by cooling with a water streamer immersed in the solution. A corex filter transmitting above 270 nm was used for photolysis.
of Ac. Progress of the reaction was monitored by thin layer chromatography. After 6 h of irradiation, removal of solvent in a rotary evaporator (30°C) and silica gel column chromatography (chloroform : methanol) of the photolysate yielded compounds 2, 3 and 4 as products. The above photoreaction was also examined in deuterium oxide and in the additive presence of sodium azide (1.0 mM), for establishing the involvement of singlet oxygen in this photoreaction.

**Potassium Ferrioxalate Actinometry**

The amount of ferrous produced is measured via spectrophotometric determination of its 1,10-phenanthroline complex at 510 nm. Ferric apparently forms only a weak complex with 1,10-phenanthroline, and this complex is transparent at 510 nm.

\[
2\text{Fe}^{3+} + \text{C}_2\text{O}_4^{2-} \xrightarrow{hv} 2\text{Fe}^{2+} + 2\text{CO}_2
\]

**Preparation of Actinometer solution**

1. Fe\(_2\)(SO\(_4\))\(_3\) solution (0.2 mol/L) was titrated with standardized EDTA using 0.2 gm salicylic acid/100 mL solution as indicator and buffered with 0.3 gm glycine/100 mL solution, to a pH of 3-4.

2. A 100 mL solution of K\(_2\)C\(_2\)O\(_4\) was prepared in such a way that its molarity is six times that of the Fe\(_2\)(SO\(_4\))\(_3\) solution (approximately 1.2 mol/L K\(_2\)C\(_2\)O\(_4\)).

3. When actinometer solution was needed, 5mL of Fe\(_2\)(SO\(_4\))\(_3\) solution and 5 mL of K\(_2\)C\(_2\)O\(_4\) solution was pipetted into a 100 mL volume flask and diluted to the mark with water.
**Intensity Measurement**

a) A volume of K$_3$Fe(C$_2$O$_4$)$_3$ solution equal to that of the samples to be irradiated was pipetted into the reaction vessel.

b) Irradiated for an appropriate period of time.

c) Irradiated solution was mixed thoroughly and an aliquot (1mL) of the actinometer was pipetted into a 10 mL volumetric flask.

d) 2 mL of 0.2 % 1,10-phenanthroline solution was added to this.

e) A volume of buffer equal to one half of the aliquot of the actinometer taken was added and diluted to the mark with water.

f) A blank was prepared by following the steps c-e with a non irradiated volume of actinometer equal to the aliquot of irradiated sample withdrawn.

g) The absorbances of the solutions ‘e’ and ‘f’ were measured vs. water at 510 nm and difference was taken.

*Calculation of light intensity:* using the absorbance obtained the light intensity was calculated from the following formula.

\[
I \text{ (einstein/min)} = \frac{AV_3V_3}{\varepsilon d \phi t V_1}
\]

Where

- $A$ Absorbance (at 510 nm) of the irradiated actinometer solution corrected for absorption of blank. (0.831)
- $d$ Path length of the absorption cell used in measurement of $A$ (1cm).
\( \varepsilon \) Extinction coefficient of ferrous 1, 10-phenanthroline complex at 510 nm 
\( (1.11 \times 10^4 \text{ L/mol/cm}) \).

\( \varphi \) Quantum yield of ferrous production at wavelength of light used. (1.03)

\( V_1 \) Volume (in milliliters) of irradiated actinometer solution withdrawn.

\( V_2 \) Volume (in liters) of actinometer irradiated. (0.20 L)

\( V_3 \) Volume (in milliliters) of volumetric flask used for dilution of irradiated aliquot (10 mL).

\( t \) irradiated time (150 min).

**Characterization of products**

*(2-Hydroxyethoxy) methyl spiroiminodihydantion (2)*: yield: 85.50 mg (38%); HRMS calcd. for \((M^+) C_8N_5O_3H_{11}\) 257.2054, found 257.2057; IR (KBr) : 3490, 3475, 3365, 3320, 1800, 1760, 1735, 1700, 1150 cm\(^{-1}\); \(^1\)H-NMR (DMSO, \( \delta \), ppm): 8.43, 8.38, 8.35, 8.27 (4H, NH), 5.24 (d, 2H), 3.71 (t, 2H), 3.56 (t, 2H); \(^1^3\)C-NMR (DMSO, \( \delta \), ppm) : 181.9 (C-8), 172.5 (C-4), 171.3 (C-6), 165.8 (C-2), 85.1 (C-5), 69.7, 67.9, 61.1; FAB-MS m/z: 258 [C\(_8\)H\(_{11}\)N\(_5\)O\(_3^+\)H]\(^+\), 184 [C\(_5\)H\(_5\)N\(_5\)O\(_3^+\)H]\(^+\).

*4-[(2-Hydroxyethoxy) methyl amino]-2-imino-1,2-dihydroimidazole-5-one (3)*: yield: 20.25 mg (9%); HRMS calcd. for \((M^+) C_{10}H_{10}N_4O_3\) 186.1700, found 186.1698; IR (KBr) : 3240, 1734, 1645, 1528, 1155 cm\(^{-1}\); \(^1\)H-NMR (DMSO, \( \delta \), ppm): 9.08 (2-NH), 8.90 (3-NH), 9.31 (5-NH), 4.73 (d, 2H), 3.70 (t, 2H), 3.56 (t, 2H); \(^1^3\)C-NMR (DMSO, \( \delta \), ppm) : 184.8 (C-2), 176.7 (C-4), 166.5 (C-5), 69.8, 69.2, 61.1; FAB-MS m/z: 187 [C\(_8\)H\(_{10}\)N\(_4\)O\(_3^+\)H]\(^+\), 113 [C\(_3\)H\(_4\)N\(_4\)O\(_2^+\)H]\(^+\).
2,2-Diamino-4-[(2-hydroxyethoxy)methyl] amino]-5-[2H]-oxazolone (4):
yield: 24.75 mg (11%); HRMS calcd. For (M⁺) C₆H₁₂N₄O₄ 204.1840, found,
204.1845; IR (KBr) : 3350, 2945, 1780, 1735, 1659, 1490, 1160 cm⁻¹; ¹H-NMR
(DMSO, δ, ppm): 7.58 (NH₂, 4H), 8.20 (4-NH), 4.73 (d, 2H), 3.70 (t, 2H), 3.56
(t, 2H); ¹³C-NMR (DMSO, δ, ppm) : 166.3 (C-2), 156.1 (C-4), 160.1 (C-5),
69.9, 69.8, 61.1; FAB-MS m/z: 205 [C₆H₁₂N₄O₄+H]⁺, 131 [C₃H₆N₄O₂+H]⁺, 161
[C₆H₁₂N₄O₄-CO₂+H]⁺, 87 [C₃H₆N₄O₂-CO₂+H]⁺.

Results and discussion

Irradiation of air saturated aqueous solution of Ac (1) in pH 7 phosphate buffer
with corex filtered light followed by purification of crude product by silica gel
column chromatography, afforded three major products, which were identified
by their spectral studies as: (2-hydroxyethoxy) methyl spiroiminodihydantion
(2). 4-[(2-hydroxyethoxy) methyl amino]-2-imino-1,2-dihydroimidazole-5-one
(3), and 2,2-diamino-4-[(2-hydroxyethoxy)methyl] amino]-5-[2H]-oxazolone
(4) (Scheme 2A.1). The study was supplemented by irradiation in the presence
of rose bengal, whereby same products were obtained, with considerably
greater conversion in a much shorter time. When rose bengal was replaced with
silica bound rose bengal¹¹ the rate of photooxidation of Ac was slower but it
contributed to a clean workup.
A brown colouration was observed for compound 4 after spraying the silica gel TLC plates with the hydroxylamine iron (III) chloride, suggesting the presence of lactone moiety in compound 4. The photomediated transformation of Ac involves singlet oxygen as evidenced from following observations: (1) No loss of substrate was observed when oxygen was excluded from medium; (2) Destruction of substrate was quenched in presence of sodium azide, a singlet oxygen quencher; (3) Loss of substrate was accelerated in D$_2$O, a well known
singlet oxygen life time promoter; (4) The enhanced degradation in D₂O was also inhibited by sodium azide.

A comparison of \(^1\)H-NMR and \(^{13}\)C-NMR spectra of Ac and those of photoproducts 2, 3 and 4 did not show any significant change in the chemical shifts of protons and carbon atoms of side chain. The slight up field shift observed for methylene protons of side chain with respect to Ac may be explained by loss of aromaticity of heterocyclic ring. The lack of H-8 resonance signal (δ 8.05) in the low field region of the \(^1\)H-NMR spectrum indicates that purine ring of the substrate has been modified. In the \(^{13}\)C-NMR spectrum of compound 2 signal at δ 181.9 was assigned to imine type carbon C-8 and signals at δ 165.8, 172.5 and 171.3 ppm were attributed to carbonyl carbon at C-2, C-4 and C-6 respectively. A significant feature of \(^{13}\)C-NMR spectrum is the appearance of a new resonance signal at δ 85.1 ppm, which was assigned to the quaternary carbon in the spiro ring in comparison with the spectra of related spirohydantoins.\(^{12,13}\) The presence of hydantoin ring in product 2 is evidenced from its infrared spectrum, which showed a sharp absorption band at 1800 cm\(^{-1}\) along with three intense absorption bands in the region 1780-1700 cm\(^{-1}\).\(^{14}\)

Inspection of the low field region of the \(^{13}\)C-NMR spectra of both the photoproducts, 3 and 4, revealed the loss of two carbon atoms in the starting compound. Resonance signals at δ 8.20 for 4 and δ 9.31 for 3, exchangeable with D₂O, were assigned to those of 4-NH for 4 and 5-NH for 3, respectively. This observation is consistent with opening of imidazole ring of Ac. In case of product 3 two other exchangeable protons observed at δ 9.08 and 8.90 were
assigned to 2-NH and 3-NH respectively. In the spectrum of 4, a broad signal at δ 7.58 equivalents to four exchangeable protons of two amino groups on sp$^3$ carbon C-2.

The structure of three products identified in this study is also well supported from their mass spectra. Base peak at m/z 258 corresponds to [C$_8$H$_{11}$N$_2$O$_5$ +H]$^+$ ion of the oxidation product 2 and peak at m/z 184 corresponds to protonated spiroiminodihydantoin, the expected consequence of fragmentation involving the loss of (hydroxyethoxy)methyl unit. The spectrum of compound 3 exhibits a major peak corresponding to protonated molecule [C$_6$H$_{10}$N$_4$O$_3$+H]$^+$ at m/z 187 and other peak at m/z 113 [C$_3$H$_6$N$_4$O+H]$^+$ arising from loss of (hydroxyethoxy) methyl unit followed by protonation. The mass spectrum of compound 4 recorded the presence of molecular ion [C$_6$H$_{12}$N$_4$O$_4$+H]$^+$ at m/z 205 and fragmentation ion [C$_3$H$_6$N$_4$O$_2$+H]$^+$ at m/z 131, arising from splitting of side chain. The two other fragments [C$_6$H$_{12}$N$_4$O$_4$–CO$_2$+H]$^+$ at m/z 161 and [C$_3$H$_6$N$_4$O$_2$–CO$_2$+H]$^+$ at m/z 87 may be rationalized by the release of CO$_2$ from the molecular and fragment ion, respectively. A similar mass fragmentation pattern has already been described for imidazolone-2'-deoxyribonucleoside and oxazolone-2'-deoxyribonucleoside.$^{15,16}$ The three products which were identified in this study as, spiroiminodihydantoin (2), imidazolone (3) and oxazolone (4) are analogous in structure to the products described in photooxidation of deoxyguanosine (dGuo) and its related derivatives.$^{15-19}$

The formation of photoproducts 2, 3 and 4 has been realized as depicted in scheme 2A.2. The reaction of dienophilic $^1$O$_2$ with guanine moiety of Ac (1) by
a Diels-Alder [4+2] reaction involving C-4 and C-8 carbons of the purine ring results in the formation of 4,8-purine endoperoxide (1a) which isomerizes to 8-hydroperoxyderivative (5). This resulting hydroperoxide may undergo dehydration followed by hydrolytic cleavage to form spiroiminodihydantoin 2 (Scheme 2A.2). Additionally, the hydroperoxide 5 in its reduced form 6 undergoes further [2+2] cycloaddition to produce an unstable dioxetane, which on subsequent decomposition gives imidazole 3 as product (Scheme 2A.3). The sequential formation of imidazolone from unstable dioxetane has its precedence in similar singlet oxygen photooxidation of nucleosides. The initially generated photoproduct, namely the imidazolone (3), on hydrolysis leads to the formation of oxazolone derivative 4 (Scheme 2A.4).
Scheme 2A.2
Scheme 2A.3
Scheme 2A.4
[A]. 2. Photooxidation of acyclovir by thermally generated triplet
excited ketone from 1,2-dioxetane and its comparison with
type I and type II photosensitizers

Biological photosensitization reactions are generally considered as belonging to either
the type I (radical mediated) or type II (singlet oxygen mediated). There are many
exogenous photosensitizers which impart differential sensitization mechanisms e.g.
riboflavin, benzophenone are predominantly type I photosensitizer and rose bengal and
methylene blue are type II, whereas 3-hydroxymethyl-3,4,4-trimethyl-1,2-dioxetane
(HTMD) is neither a typical type I nor a characteristic type II photosensitizer. Hence,
in an extensive study, photooxidation of acyclovir was investigated by thermally
generated triplet-excited ketone from HTMD for its comparison with type I and type II
photosensitizers. The triplet excited ketones are important class of photooxidative
sensitizers of biological interest since they may be generated in cellular systems upon
exposure of endogenous chromophores to UV radiation or by dark reactions.

Thermal decomposition of HTMD, which is a convenient chemical source of triplet-
excited ketone, is accompanied by infrared photoemission at 1270 nm, characteristic
for singlet oxygen monomolecular emission, and thus HTMD are known to generate
singlet oxygen in sequence, and can operate as type I or type II photooxidants.

The photooxidation of Ac was conducted in presence of thermally generated triplet
excited ketone. The distribution of the products was compared with those of sensitized
photooxidation of Ac by riboflavin (type I) and rose bengal (type II).
Experimental

Reaction procedure

For the HTMD (prepared as per literature\textsuperscript{28}) mediated oxidation of Ac, a 0.5 mM solution of Ac in 10 mM sodium cacodylate buffer (pH 7.0) and 10 vol% of dioxetane solution in acetonitrile was kept at 50 °C for 18 h in absence of light. The photosensitized oxidation of phosphate buffered solution of Ac (0.5 mM) was carried out in the presence of photosensitizer riboflavin or rose bengal for 2.5 h by irradiation with a 150 W sodium lamp. 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. After the given time period the amount of photoproducts formed and the remaining amount of Ac was determined by isolation and purification of the reaction mixture using silica gel column chromatography.

Relative yields of the products was determined on the basis of consumed Ac and the mean value of at least 3 independent run, and a comparison of the results with the different sensitizers is presented in Table 2A.1. The concentration and time profile for HTMD induced oxidation of Ac (0.5 mM) at 50 °C was also determined by using 10 mM sodium cacodylate buffer (pH 7) as a reaction medium with 10 vol% of acetonitrile as co-solvent. Effect of D\textsubscript{2}O on the yield of Sp in HTMD-induced photooxidation was 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 obtained. The products were identified as (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-[2H]-oxazolone (4) (Scheme 2A.5). The photoproducts were identified by comparing their spectral data with that of singlet oxygen mediated Ac photooxidation products spiroiminodihydantoin (Sp) imidazolone (Im) and oxazolone (Ox). Similar products pattern was obtained when Ac was irradiated in an aerated aqueous solution containing riboflavin or rose bengal, but different yields of products were obtained depending upon the photosensitizer used. (Table 2A.1) 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 close to each other. The Sp/Im+Ox product ratio indicating the relative predominance of photooxidation products was taken as mechanistic probe to assess the extent of type I and type II photosensitized oxidation. 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%).
Energy transfer

Electron transfer

Scheme 2A.5
Table 2A.1 Product balance of Ac oxidation by dioxetane HTMD and by riboflavin and rose bengal photosensitizers. *Relative yields based on consumed Ac; mean value of three independent runs.
This difference in products distribution may be accounted for by the fact that rose bengal produce singlet oxygen in large amount by a type II mechanism, whereas riboflavin, which acts 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 Figure 2A.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 2A.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.
Figure 2A.1 Concentration profile for the thermally HTMD-induced photooxidation of Ac, yields derived from the mean values of three independent runs. (●) conversion of Ac, (▲) Yields of Ox and Im, (■) yields of Sp.
Figure 2A.2 Time profile for the thermally HTMD-induced photooxidation of Ac, yields derived from the mean values of three independent runs. (●) conversion of Ac, (▲) Yields of Ox and Im, (■) yields of Sp.
The linear increase of Ac conversion with increasing HTMD concentration suggested that the HTMD oxidation of Ac is directly proportional to the amount of triplet-excited ketone 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 ketone.

Proof for the involvement of singlet oxygen in HTMD-induced oxidation came from the substantial effect of D$_2$O on the formation of Sp. Higher yields of Sp in D$_2$O compared to those of H$_2$O indicated that singlet oxygen is involved in HTMD mediated photooxidation. Singlet oxygen quenchers such as DABCO, sodium azide etc. could not be used to confirm the involvement of singlet oxygen in HTMD mediated photooxidation as they are known to react with dioxetane.$^{29,30}$

Our present investigation revealed that triplet excited ketone generated in the thermal decomposition of HTMD oxidizes 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 were obtained as major products, whereas Sp was characterized as a major type II photooxidation product of rose bengal sensitized oxidation of Ac. The HTMD-induced photooxidation of Ac may have an implication to the in vivo photobiological transformation in dark and of significance to 'photobiology without light'.31,32
Section [B]

Photochemistry of Phenazopyridine Hydrochloride
Phenazopyridine hydrochloride (2,6-diamino-3-phenylazopyridine) (PhPy, 8) is the generic name for an azo dye, which has been used for 40 years as an analgesic drug to reduce pain, associated with urinary tract infection. Phenazopyridine with an azo chromophore is expected to be photolabile and a probable photosensitizer of biological substrates. Moreover, several drugs with phenylazo moiety are known to biometabolize to arenediazonium ion, which is known to behave as photosensitizer. Interest in the photoreactivity of phenazopyridine arises from the clinical and pharmacological reports of toxic effects associated with the use of this drug. 2,3,6-Triaminopyridine (11), a metabolite of phenazopyridine, is known to cause muscle necrosis and renal damage in rats, and it is reasonably anticipated to be human carcinogen, based on sufficient evidence of carcinogenicity in experimental animals.

In the present study we have investigated the photolysis of phenazopyridine in different reaction medium, including the drug adsorbed on silica gel, as a biological mimic of situation in liposomes. The results of photolyses are outlined in Scheme 2B.1. The photoreactivity of phenazopyridine was enhanced in the organized medium of silica gel. The various products of photolysis were fully characterized by IR, $^1$H-NMR, $^{13}$C-NMR and mass spectrometric studies. Formation of 2,3,6-triaminopyridine (11) is probably indicative of phototoxicity of drug, as it is identical with the known toxic metabolite of the drug.
Experimental

Apparatus

Same as in section [A]. 1.

Chemicals

All chemical used were of analytical grade. Phenazopyridine hydrochloride (8), was extracted from commercial medicament Pyridium (Parke Davis, India). The purity of drug extracted, was checked by TLC.

Irradiation of phenazopyridine hydrochloride in methanol

Phpy (8) 2 gm (0.009 mole) was dissolved in 100 ml methanol and irradiated in a photoreactor equipped with a 450 W medium pressure mercury vapour lamp inserted in a water cooled quartz fitted immersion well with continuous circulation of water. As the reaction progressed, solution becomes brighter. The progress of the reaction was monitored by TLC using a solvent system of chloroform-methanol (80:20) mixture. After irradiation of the mixture for 12 hours the solvent was removed in a rotary evaporator and crude product was subjected to silica gel column chromatography. Elution with chloroform-petrol gave 9, 10, 11 and 12 as products.

Pyrido[3,4-c]cinnoline-2,4-diamine (9): Yield: 35%; UV $\lambda_{max}$(MeOH) 252 and 368 nm; HRMS caled. for (M$^+$) C$_{11}$N$_5$H$_9$ 211.0880, found 211.0860; IR(KBr) : 3500, 3200, 1570, 1200 cm$^{-1}$; $^1$H-NMR (CDCl$_3$) $\delta$ 4.0 (brs, 4H, exch., aromatic C-NH), 5.92 (s, 1H, H-1), 7.57 (m, 3H, H-8, H-9, H-10), 8.30 (d, J=6.5 Hz, 1H, H-7); $^{13}$C-NMR (CDCl$_3$) $\delta$ 98.3 (C-1), 123.4, 129.6, 131.0, 138.3, 140.5, 146.1, 150 (cinnoline), 151.6 (C-4), 158.1
$N^\text{13}$-Phenylpyridine-2,3,4,6-tetraamine (10): Yield: 15%; HRMS calcd. for (M$^+$) C$_{11}$N$_3$H$_{13}$, found 215.1218; IR(KBr): 3510, 3400, 1590, 1410 cm$^{-1}$; $^1$H-NMR (CDCl$_3$) $\delta$ 4.0 (brs, 7H, exch., aromatic C-NH), 5.20 (s, 1H, H-5), 6.46 (d, J=2.5 Hz, 2H, phenyl), 6.62 (m, 1H, phenyl), 7.01 (m, 2H, phenyl); $^{13}$C-NMR (CDCl$_3$) $\delta$ 85.4 (C-5), 106.0 (C-3), 116.3, 118.8, 129.6, 143.1 (phenyl), 145.7 (C-4), 148.2 (C-6), 149.2 (C-2); MS: m/z (rel int.) M+1: 216 (7), 215 (45.1), 214 (100.0), 186 (3.9), 185 (6.7), 137 (27.1), 110 (12.7), 109 (9.14), 108 (3.1).

Pyridine-2,3,6-triamine (11): Yield: 12%; HRMS calcd. for (M$^+$) C$_5$N$_4$H$_8$, found 124.0904; IR(KBr): 2925, 1460, 1377, 1320, 763 cm$^{-1}$; $^1$H-NMR (CDCl$_3$) $\delta$ 4.0 (brs, 6H, exch., aromatic C-NH), 5.94 (d, J = 8 Hz, 1H, H-5), 6.64 (d, J = 8 Hz, 1H, H-4); $^{13}$C-NMR (CDCl$_3$) $\delta$ 100.2 (C-5), 122.0 (C-3), 125.2 (C-4), 147.3 (C-6), 148.3 (C-2); MS: m/z (rel int.) M+1: 125 (70), 108 (63), 81 (30), 54 (100).

2,6-Diamino-1-(4-aminophenyl)pyridin-4(1H)-one (12): Yield: 5%; HRMS calcd. for (M$^+$) C$_{11}$N$_4$OH$_12$, found 216.1169, IR(KBr): 3500, 3430, 1700, 1665 cm$^{-1}$; $^1$H-NMR (CDCl$_3$) $\delta$ 2.0 (brs, 4H, exch., -NH$_2$), 4.0 (brs, 2H, exch., aromatic C-NH), 4.42 (s, 2H, dienone protons), 6.21 (s, 4H, aromatic protons); $^{13}$C-NMR (CDCl$_3$) $\delta$ 82.6 (C-3 and C-5), 117.1 (C-2', 3', 5', 6'), 131.3 (C-1'), 138.4 (C-4'); MS: m/z (rel. int.) M+1: 217 (31.5), 189 (5.3), 140 (2.3), 139 (28.1), 112 (8.1), 110 (100.0).

Irradiation of Phpy (8) adsorbed on silica gel
The drug was dissolved in methanol and mixed with aqueous slurry of silica gel. TLC plates were prepared and photolyzed as such with a 450 W medium pressure mercury
lamp. The plate appeared as yellow chromatogram, which turned dark yellow within 15 min. Photolysis was continued up to 4 hours for complete decomposition of the drug. The progress of the reaction was monitored by withdrawing a scratch of irradiated silica gel and its co-TLC with the drug. Complete scratch from the plate was dissolved in acetone, filtered and evaporated in a rotary evaporator followed by chromatography on silica gel yielded 9, 10, 11 and 12 as products.

**Results and discussion**

Irradiation of methanol solution of Phpy (8) with medium pressure mercury vapour lamp in a immersion well type photoreactor gave pyrido[3,4-c]cinnoline-2,4-diamine (9), N\textsuperscript{2}-phenylpyridine-2,3,4,6-tetraamine (10), pyridine-2,3,6-triamine (11) and 2,6-diamino-1-(4-aminophenyl)pyridin-4(1H)-one (12) as photoproducts (Scheme 2B.1), which were characterized from their spectral studies. None of these products showed IR band at 1600 cm\textsuperscript{-1} typical for free azo group, however a band at 1570 cm\textsuperscript{-1} in the IR of 9 could be assigned to \(-\text{N=N-}\) in cinnoline. Photoproduct 9 showed a broad singlet at \(\delta\) 3.95 ppm equivalent to two \(-\text{NH}_{2}\) group protons. A sharp singlet at \(\delta\) 5.92 ppm was assigned to the only proton present in the diamino substituted pyridine ring. NMR signals for the aromatic ring amounting to only four protons along with characteristic UV bands at 252 and 368 nm supported for a benzo[c]cinnoline\textsuperscript{42} structure.

Product 10 showed a broad singlet at \(\delta\) 4.0 ppm due to protons of aromatic \(-\text{NH}_{2}\) group. A sharp singlet at \(\delta\) 5.2 ppm, logically upfield to the benzene ring protons, was assigned to a single proton flanked by two amino groups in the pyridine ring. This is further supported by the \(^{13}\text{C}\)-NMR value of \(\delta\) 85.4 ppm for the only unsubstituted carbon to
which this hydrogen is attached. The NMR spectrum of product 11, with a six proton broad singlet at δ 4.0 ppm for aromatic amino group and a pair of ortho coupled doublets at δ 5.94 and 6.64 ppm for aromatic protons, along with its mass spectrum established 2,3,6-triaminopyridine structure for it.
The photoproduct 12 showed two types of –NH₂ signals: at δ 4.0 for two protons and at δ 2.0 for four protons. A sharp singlet for four protons at δ 6.21 ppm indicated that aromatic ring is para-disubstituted. A β,β'-diaminodienone structure for it was supported by proton signal at δ 4.42 ppm for dienone protons and 13C-signal at δ 185.8 ppm for carbonyl carbon and additionally by IR frequency at 1700 and 1665 cm⁻¹ (C=O).

The probable course of formation of products is described in scheme 2B.2 and 2B.3. The product 9 results from photochemical cyclodehydrogenation of phenazopyridine, whereby a reduced molecule of the drug (8a) also produced.⁴² Phenhydrazopyridine (8a) undergoes reductive degradation to 2,3,6-triaminopyridine (11) and in an alternative course rearranges to N^3-phenylpyridine-2,3,4,6-tetraamine (10). The reductive degradation of 8a probably co-generates phenylnitrenium ion (Scheme 2B.2), as traces of p-methoxyaniline was detected on TLC. Arynitrenium ions are known intermediates in physiological DNA-damaging reactions, which are responsible for carcinogenesis.⁴³ 2,6-Diamino-1-4(4-aminophenyl)pyridine-4-(1H)-one (12) was proposed to be derived from 10 in a sequence shown in Scheme 2B.3.
Scheme 2B.2
Scheme 2B.3
References


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