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Photochemistry of phenazopyridine hydrochloride

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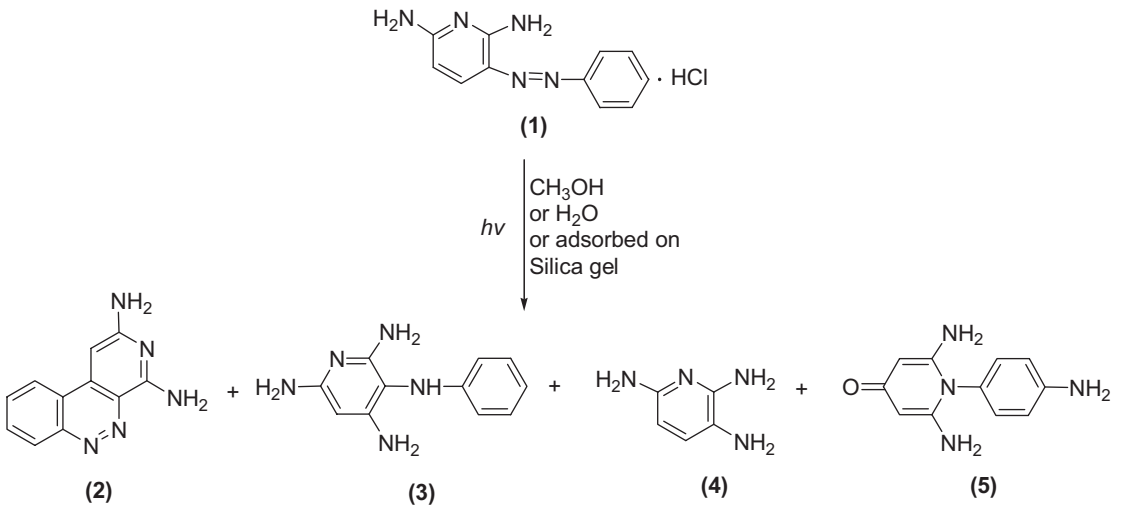
Phenazopyridine hydrochloride (**1**) is an azo dye with local analgesic and anaesthetic effects on the urinary tract. Its photochemistry was studied in different reaction media including the drug adsorbed on silica gel. This resulted in photochemical cyclodehydrogenation, reductive photodegradation and rearrangement of the drug molecule. Four major products were isolated and identified on the basis of IR, NMR and mass spectral studies. The products are: pyrido[3,4-*c*]cinnoline-2,4-diamine (**2**), N^3-phenylpyridine-2,3,4,6-tetraamine (**3**), pyridine-2,3,6-triamine (**4**), 2,6-diamino-1-(4-aminophenyl)pyridin-4(1*H*)-one (**5**).

1. Introduction

The molecular mechanism of biological photosensitization induced by drugs and their phototoxicity is receiving increasing attention (Cosa 2004; Miranda 2001; Beigersbergen van Henegouwen 1997). With regard to the mechanistic considerations basically four main pathways as routes for phototoxic reactions are known, namely singlet oxygen formation and its reaction with drug, radical formation, covalent photobinding to biomolecules and photoproducts in decomposition reaction (Quintro and Miranda 2000). Studies on the photodegradation of drugs are relevant to the drug development process, because the photolysis products may have biological effects different from those of the parent compounds.

Phenazopyridine hydrochloride (2,6-diamino-3-phenylazopyridine) (**1**) 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 infections (Doerge 1977). 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 an arenediazonium ion, which is known to behave as photosensitizer (Quintri and Miranda 2000). Interest in the photoreactivity of phenazopyridine arises from the clinical and pharmacological reports of toxic effects (Narchi and Aramco 2000; Munday and Fowke 1994) associated with the use of this drug. 2,3,6-triaminopyridine (**4**), a metabolite of phenazopyridine, is known to cause muscle necrosis and renal damage in rats (Munday and Manns 1998) and it is reasonably anticipated to be a human carcinogen (IARC 1987) based on sufficient evidence of carcinogenicity in experimental animals (IARC 1980, IARC 1982).

Scheme 1



In the present study we have investigated the photolysis of phenazopyridine in different reaction media, including the drug adsorbed on silica gel, as a biological mimic of the situation in liposomes (Yamamoto and Borch 1985). The results of photolyses are outlined in Scheme 1.

2. Investigations, results and discussion

Irradiation of methanolic solution of (**1**) with a medium pressure mercury vapour lamp in an immersion well type photoreactor gave pyrido[3,4-*c*]cinnoline-2,4-diamine (**2**), N^3-phenylpyridine-2,3,4,6-tetraamine (**3**), pyridine-2,3,6-triamine (**4**), 2,6-diamino-1-(4-aminophenyl)pyridin-4(1*H*)-one (**5**) as photoproducts (Scheme 1), which were characterized from their spectral studies.

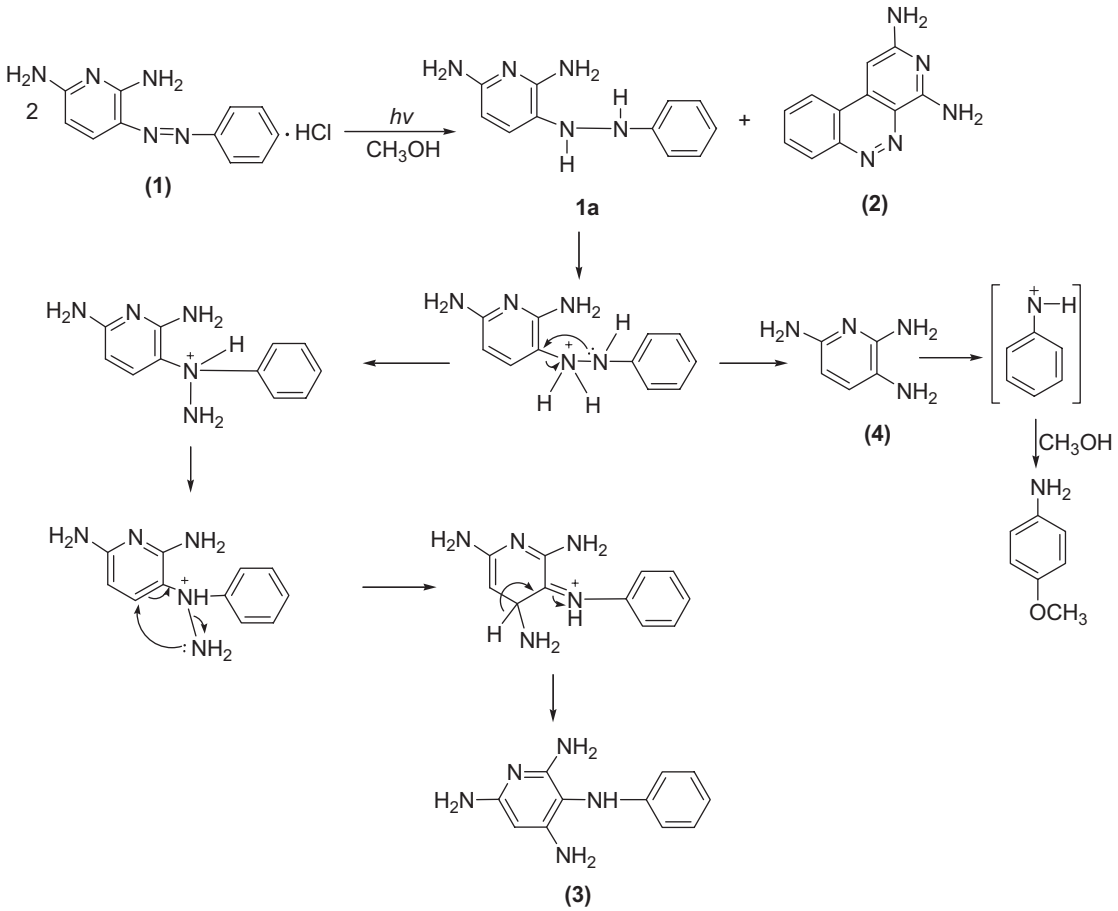
None of these products showed an IR band at 1600 cm^{-1} typical for free azo group, however a band at 1570 cm^{-1} in the IR of **2** could be assigned to $-N{=}N-$ in cinnoline. Photoproduct **2** showed a broad singlet at δ 3.95 ppm equivalent to two $-NH_2$ group protons. A sharp singlet at δ 5.92 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 (Badger et al. 1963) structure.

Product **3** showed a broad singlet at δ 4.0 ppm due to protons of aromatic $-NH_2$ group. A sharp singlet at δ 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}C NMR value of δ 85.4 ppm for the only unsubstituted carbon to which this hydrogen is attached. The NMR spectrum of product **4**, 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 a 2,3,6-triaminopyridine structure for it.

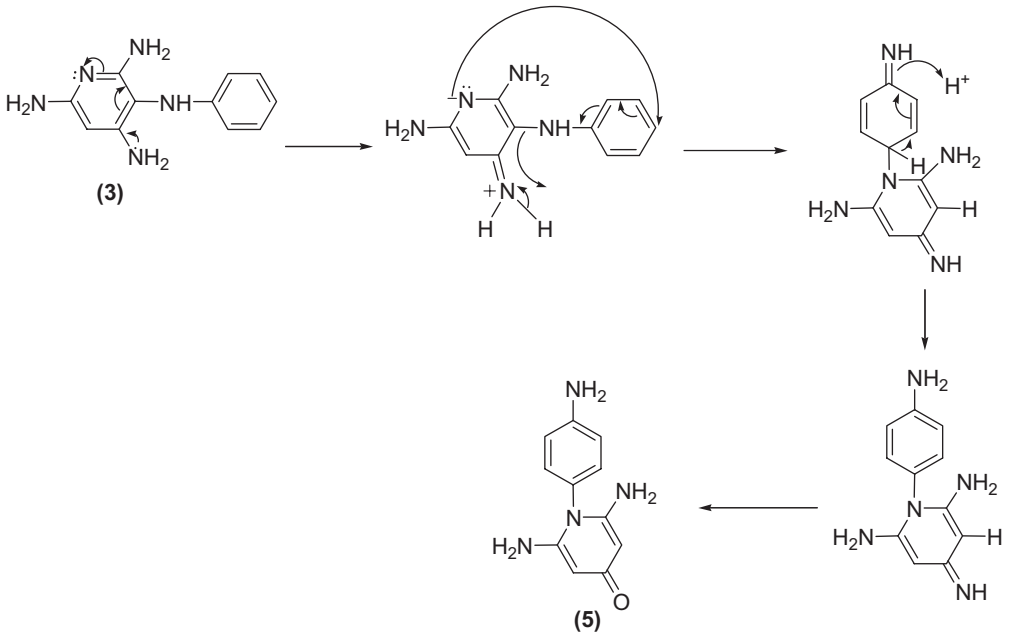
The photoproduct **5** showed two types of $-NH_2$ signals: at δ 4.0 for two protons and at δ 2.0 for four protons. A sharp singlet for four protons at δ 6.21 ppm indicated that the aromatic ring is para-disubstituted. Its β,β′-diaminodienone structure was supported by a proton signal at δ 4.42 ppm for dienone protons and a ^{13}C-signal at δ 185.8 ppm for carbonyl carbon and additionally by an IR frequency at 1700 and 1665 cm^{-1} (C=O).

The probable course of formation of products is shown in Schemes 2 and 3. Product **2** results from photochemical cyclodehydrogenation of phenazopyridine, whereby a reduced molecule of the drug (**1a**) was also produced (Badger et al. 1963). Phenhydrazopyridine (**1a**) undergoes reductive degradation to **4** and in an alternative course rearranges to **3**. The reductive degradation of **1a** probably co-generates a phenylnitrenium ion, as traces of *p*-methoxyaniline were detected by TLC (Scheme 2). Arylnitrenium ions are known as intermediates in physiological DNA-damaging reactions, which are responsible for carcinogenesis (Falvey et al. 1996). 2,6-Diamino-1–4(4-aminophenyl)pyridine-4-(1*H*)-one (**5**) was proposed to be derived from **3** in a sequence shown in Scheme 3.

Scheme 2



Scheme 3



3. Experimental

3.1. Apparatus

Photochemical reactions were carried out in a quartz fitted immersion well photochemical reactor equipped with 400 W medium pressure mercury vapour lamp with continuous supply of water. The incident photon flux of the irradiation setup was 8.72×10^{-7} einstein/min as determined by using ferrioxalate actinometry (Hatchard and Parker 1956). UV spectra were recorded on a Shimadzu 160 A instrument. IR spectra were recorded in 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 were obtained on a VG-ZAB-HS mass spectrometer. High-resolution mass spectra were determined with a VG-ZAB-BEQ9 spectrometer at 70 eV ionization voltage. Column chromatography was performed on silica gel 60 (70–230 mesh). TLC was carried on Merck silica gel 60 F_{254} (0.2 mm-thick plates).

3.2. Chemicals

All chemicals used were of analytical and pharmaceutical grade. Phenazopyridine hydrochloride (**1**), was extracted from the commercial medicament Pyridium (Parke Davis, India). The purity of drug extracted was checked by TLC and comparing its melting point with the literature value.

3.3. Irradiation of phenazopyridine hydrochloride in methanol

Phenazopyridine hydrochloride (1, 2 g, 0.009 mol) was dissolved in 100 ml methanol and irradiated in the photoreactor (3.1). As the reaction progressed, solution became brighter. The progress of the reaction was monitored by TLC using a solvent system of chloroform-methanol mixture. After irradiation of the mixture for 12 h the solvent was removed in a rotary evaporator and crude product was subjected to silica gel column chromatography. Elution with chloroform-petrol gave **2**, **3**, **4** and **5** as products.

Compound ***2***: Yield: 35%; UV λ_{max}(MeOH) 252 and 368 nm; HRMS calcd. for (M^+) $C_{11}N_5H_9$ 211.0880, found 211.0860; IR(KBr): 3500, 3200, 1570, 1200 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.0 (brs, 4 H, exch., aromatic C–NH), 5.92 (s, 1 H, 1-H), 7.57 (m, 3 H, 8-H, 9-H, 10-H), 8.30 (d, J = 6.5 Hz, 1 H, 7-H); ^{13}C NMR ($CDCl_3$) δ 98.3 (C-1), 123.4, 129.6, 131.0, 138.3, 140.5, 146.1, 150 (cinnoline), 151.6 (C-4), 158.1 (C-2); MS m/z (rel. int.) M + 1: 212 (100), 214 (12.1), 213 (54.7), 184 (4.9), 183 (5.7), 136 (29.6).

Compound ***3***: Yield: 15%; HRMS calcd. for (M^+) $C_{11}N_5H_{13}$ 215.1218, found 215.1215; IR(KBr): 3510, 3400, 1590, 1410 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.0 (br s, 7 H, exch., aromatic C–NH), 5.20 (s, 1 H, 5-H), 6.46 (d, J = 2.5 Hz, 2 H, phenyl), 6.62 (m, 1 H, phenyl), 7.01 (m, 2 H, phenyl); ^{13}C NMR ($CDCl_3$) δ 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).

Compound ***4***: Yield: 12%; HRMS calcd. for (M^+) $C_5N_4H_8$ 124.0904, found 124.0901; IR(KBr): 2925, 1460, 1377, 1320, 763 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.0 (brs, 6 H, exch., aromatic C–NH), 5.94 (d, J = 8 Hz, 1 H, 5-H), 6.64 (d, J = 8 Hz, 1 H, 4-H); ^{13}C NMR ($CDCl_3$) δ 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).

Compound ***5***: Yield: 5%; HRMS calcd. for (M^+) $C_{11}N_4OH_{12}$ 216.1169, found 216.1171; IR(KBr): 3500, 3430, 1700, 1665 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.0 (brs, 4 H, exch., $-NH_2$), 4.0 (brs, 2 H, exch., aromatic C–NH), 4.42 (s, 2 H, dienone protons), 6.21 (s, 4 H, aromatic protons); ^{13}C NMR ($CDCl_3$) δ 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).

3.4. Irradiation of 1 adsorbed on silica gel

The drug was dissolved in methanol and mixed with aqueous slurry of silica gel. TLC plates were prepared and wet plate photolyzed as such with a 400 W medium pressure mercury lamp. The plate appeared as yellow chromatogram, which turned dark yellow within 15 min, Photolysis was continued up to 4 h for complete decomposition of drug. The progress of reaction was monitored by withdrawing a scratch of irradiated silica gel and it's Co-TLC with the starting drug. The complete scratch from the plate was dissolved in acetone, filtered and evaporated in a rotatory evaporator followed by chromatography on silica gel yielded **2**, **3**, **4** and **5** as products.

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