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PHOTOCHEMISTRY OF PHOTODYNAMIC BIOLOGICAL ACTION OF PHOTOSENSITIZING DRUG TOLAZAMIDE

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ABSTRACT

Irradiation of sulfonamide derived oral antidiabetic drug, tolazamide (N-[(azepan-1-ylamino) carbonyl]-4-methylbenzenesulfonamide, 1) in a phosphate buffered solution under aerobic atmosphere afforded two photoproducts by cleavage of S-N and C (O)-N bonds of urea units. When irradiated with linoleic acid it photoinduced lipid peroxidation. The insignificant decrease in lipid peroxidation test under argon atmosphere indicates that tolazamide is capable of photosensitizing lipids through a process where oxygen does not play a principal role. Also the efficient inhibition of lipid peroxidation by well established radical scavenger reduced glutathione (GSH) confirmed the involvement of a type I mechanism.

KEY WORDS: Tolazamide, Benzene sulfonamide, Photodegradation, Phototoxicity.

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INTRODUCTION

The molecular mechanism of biological photosensitization induced by drugs and their phototoxicity is receiving increasing attention.[1, 2] With regard to the mechanistic considerations basically four pathways as routes for phototoxic reactions are known namely singlet oxygen formation and its reaction with drug, radical formation, and covalent photobinding to biomolecules and photoproduct in decomposition reaction.[3, 4] The number and variety of phototoxic compound is large. This, together with the limited research effort devoted to this subject so far, means that for most phototoxic xenobiotics a relationship between structure and photoreactivity is not easily found. [5, 6] It is assumed that there must be an appropriate correlation between photochemical behaviour and phototoxicity, and it is therefore necessary to investigate the photochemistry of every photosensitizing drug. The sulfonamides have, for many years, being widely studied for their chemotherapeutic activity. Their important role as antibacterial, antimalarial and antileprotic agents is well recognized[7]. The sulfonamide group is considered as chromophore which is present in a number of biologically active molecules, particularly in antimicrobial agents[8-10]. In addition, numerous sulfonamide derivatives have been reported as carbonic anhydrase inhibitors[11-14], anti-cancer[15], and anti-inflammatory agents[16]. Benzene derivatives of sulfonamides called benzene sulfonamide have attracted much consideration due to their chemical and pharmacological applications. Some benzene sulfonamide are important clinical agents, mainly they are used in the treatment of gastro-intestinal-duodenal ulcers, neurological disorders, glaucoma, altitude sickness and for some forms of tumor.[17,18] The therapeutic use of benzene sulfonamide derived drugs as antidiabetes agent has been associated in some patients with the appearance of the phototoxic effects such as erythema, flaring and urticarial weal. In vitro experiments also reveal the potential phototoxicity of these drugs[19]. These compounds have been recognized as a very good photo sensitizer in clinical test and in cell culture.[20] Although it is a very useful but it can produce adverse biological effects such as clinical photosensitization which occurs on the skin of patients. [21] Tolazamide is a benzene sulfonamide derivative and it is an oral hypoglycemic agent of the aryl sulphonylurea type, [22] similar to tolbutamide chloropromamide and acetohexamide. It is approximately five times more potent than tolbutamide in the human diabetic [23, 24]. Tolazamide with sulfonamide chromophores is expected to be photolabile and probable photo sensitizer of biological substrate. Interest in the photochemistry of tolazamide arises from the clinical and pharmacological reports of phototoxic effects associated with the use of this drug [25, 26]. In this study, our goal was to characterize the photochemical properties of tolazamide, in order to understand and rationalize the basic photochemical reaction involved in the phototoxicity. Photolysis of tolazamide (1) in the presence of oxygen resulted in the formation of two photodegradation products, identified as 2 and 3 from their spectral (IR, ^{1}H-NMR, ^{13}C-NMR, mass spectra) properties (Scheme-1).Product 2 and 3 presumably produced by the rupture of CO-N bond of tolazamide (1).

EXPERIMENTAL

Apparatus and Chemicals

All chemicals used were of analytical and pharmaceutical grade. Tolazamide (1) was extracted from the commercial medicament Tolinase (Pfizer, India). The purity of drug, extracted was checked by thin layer chromatography (TLC) and comparing its melting point with the literature value. Rose bengal and reduced glutathione (GSH) were purchased from Sigma Aldrich (India). Photochemical reactions were carried out in quartz fitted immersion well photochemical

reactor equipped with 400W medium pressure mercury vapour lamp with continuous supply of water. UV spectra were recorded on a Shimadzu 160 A Instrument. IR spectra were recorded in KBr discs on a Perkin Elmer model spectrum RXI. ^{1}H-NM R and ^{13}C-NM R Spectra were recorded on a Bruker Avance-DRX-300 Spectrometer using $SiMe_4$ as internal standard and $CDCl_3$ as solvent. E/MS were obtained on a VG-ZAB-HS mass spectrometer. High resolution mass spectra were determined with a VG-ZAB-BEQ9 spectrometer at 70 e V 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).

Irradiation Procedure

Tolazamide (1) 275 mg (0.8 mM) was dissolved in 400 ml methanol and irradiated at room temperature in the Rayonet photochemical reactor. Progress of the reaction was monitored by thin layer chromatography (chloroform-methanol, 98:2). After the irradiation of mixture for 71 hrs the solvent was removed in a rotary evaporator and the crude product was subjected to silica gel column chromatography elution with CH_3CN/H_2O (30:70, v/v) on silica column gave 2 and 3 as products.

Azepan-1-amine (2):

Yield: 65 mg (23.6 %); UV λ_{max} (MeOH) 238 nm and 242 nm; HRMS calcd. for (M^+) $C_6H_{14}N_2$ 114.1888, found 114.1757; IR (KBr): 3140, 3250 (NH_2), 2880, 2967, cm^1; ^{1}HNMR ($CDCl_3$, δ, ppm): 2.57 (t, J=7.1 Hz, 4H, H-2 & H-7), 2.0 (s, 2H, NH_2), 1.42 (m, 4 H, H-3 & H-6), 1.28 (m, 4H, H-4 & H-5); ^{13}C-NMR ($CDCl_3$, δ, ppm): 61.1 (C-2 & C-7), 26.5 (C-4 & C-5), 25.5 (C-3 & C-6); MS: m/z: 114 (M^+), 98 (M^+- NH_2).

N-tosylformamide (3):

Yield: 98 mg (35.6 %); UV λ_{max} (MeOH) 261 nm and 210 nm; HRMS calcd. for (M^+) $C_8H_9NO_3S$ 199.2270 found 199.2268; IR (KBr): 3359, 3325(NH), 1655 (CONH), 1340, 1160 cm^{-1} (SO_2); ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.75 (s, 1H, CHO), 7.82 (d, J=7.9 Hz , 2H, tolyl H-3 and H-5), 7.32 (d, J=7.9 Hz, 2H, tolyl H-2 and H-6), 2.38 (s, 3H, tolyl CH_3), 2.0 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, δ, ppm): 175.01 (CO, CHO), 142.6, 136.9, 129.8 128.3, 23.4, (C-1, C-4, C-2 and C-6, C-3 and C-5, CH_3 of the toluene moiety); MS: m/z: 199 (M^+), 185 (M^+- CH_3), 170 (M^+- HCO), 155 (M^+-HCONH), 92 (M^+-$HCONHSO_2$).To observe a possible quencher effect of the tolazamide on singlet oxygen ($^1O_2$), tolazamide (1) was also irradiated in the presence of photosensitizer rose bengal and maintaining all other experimental conditions the same.

Photosensitized per oxidation of linoleic acid

Phosphate buffered solution of Linoleic acid (1×10^{-3} M) was irradiated in the presence of compound 1 and also in a pre-irradiated solution of 1 (1×10^{-5}). The formation of dienic hydroperoxides was monitored by UV spectrophotometry, by the appearance and progressive increases of a new band at λ=233 nm (fig-1) [27]. The lipid peroxidation test was repeated in the presence of reduced glutathione (GSH, a radical scavenger). This test was also carried under argon atmosphere.

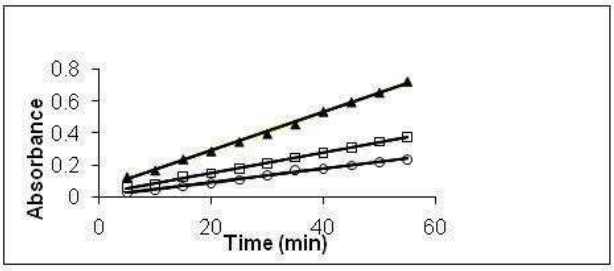
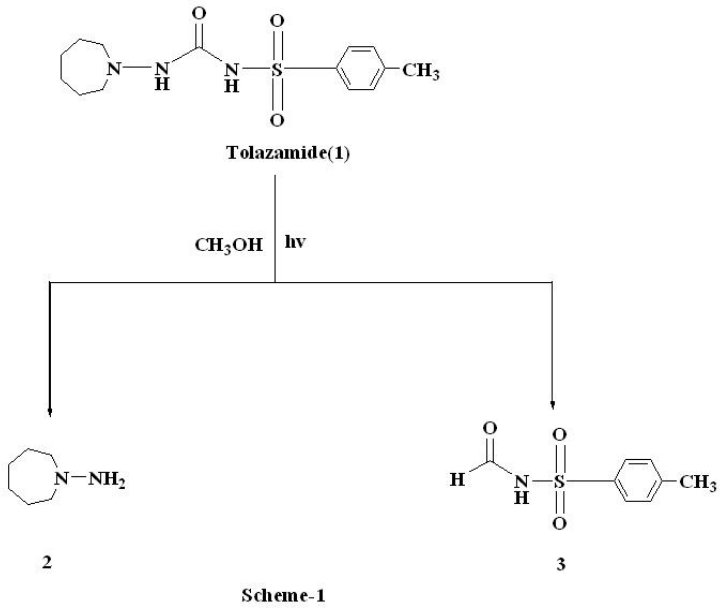


Figure 1
Photoperoxidation of linoleic acid (L, 10^{-3}M) sensitized by Tolazamide (1)

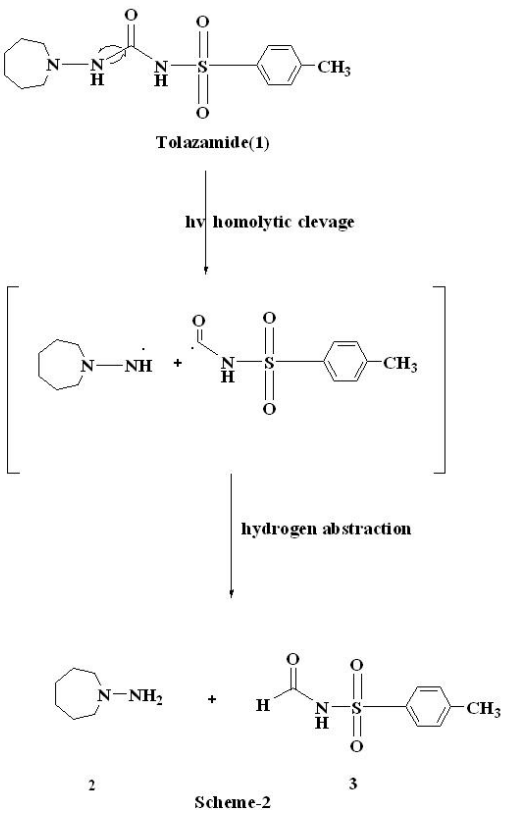
RESULTS AND DISCUSSION

Irradiation of methanolic solution of tolazamide (1) under oxygen atmosphere with a medium pressure mercury vapour lamp in an immersion well type photo reactor gave Azepan-1-amine (2), N-tosylformamide (3) as photo products (scheme-1) which were characterized from their spectral studies. No degradation of tolazamide was observed when irradiation was carried out in the presence of rose bengal. Therefore, the interaction with or quenching of singlet oxygen ($^1O_2$), by tolazamide was negligible. Tolazamide undergoes homolytic cleavage (rupture of) CO-NH bond resulting in the generation of two intermediate radicals (2a) and (3a) (scheme-2). Hydrogen abstraction by intermediate radicals generated in tolazamide photodegradation leads to lipid peroxidation (Scheme-3).

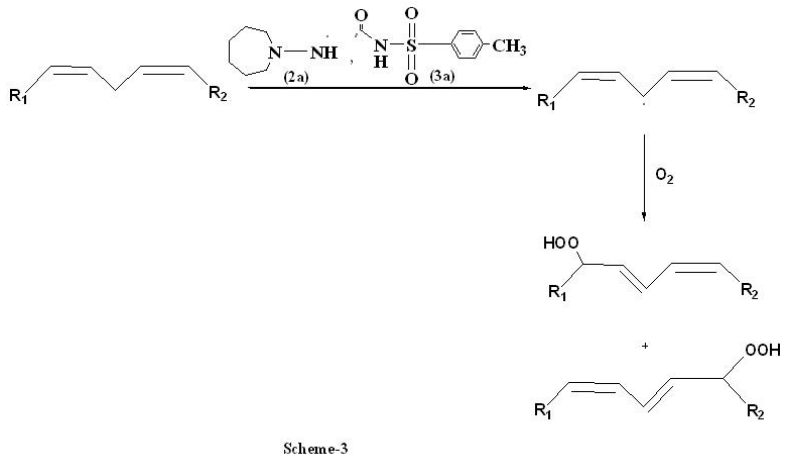


Photodegradation products of tolazamide

The efficient inhibition of lipid peroxidation by well-established radical scavengers GSH, confirmed the involvement of type I mechanism. The phototoxicity mechanism for tolazamide most probably involves reaction of free radical intermediates and photoproducts with cellular components. The insignificant decrease in lipid peroxidation test under argon atmosphere indicates that tolazamide is capable of photosensitizing lipids through a process where oxygen does not play principal role.



Mechanistic pathway of photodegradation of tolazamide



Photoperoxidation of linoleic acid sensitized by tolazamide

The studies of phototoxicity carried out in this work may help to explain the damage produced in protein and organs. The observations in this work may contribute to elucidate the observed accumulations and damaging activity of oxidized proteins during aging and in pathologies such as diabetes, atherosclerosis and neurodegenerative diseases. Lipid photoperoxidation certainly correlates the damage produced in the cell membranes. The phototoxicity mechanism for tolazamide most probably involves reaction of free radical species than singlet oxygen, super oxide anion or photo products with cellular components. The result obtained may be very useful from medical point of view to perform the appropriate screening of phototoxicity in vitro before introducing drugs and chemicals into chemical therapy.

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