Wavelength Dependency and Mechanism of the Photo-Degradation of Ethyl 2-[4,5-Bis(4-methoxyphenyl)thiazole-2-yl]pyrrol-1-ylacetate (KBT-3022) in Solution[1)]

Tsunemasa MIZUNO,*,[a] Masahiro HANAMORI,[a] Koichi AKIMOTO,[a] Hiroshi NAKAGAWA,[a] and Katsumasa ARAKAWA[b]

Pharmaceuticals Research Center, Kanebo, Ltd.,[a] 1–5–90, Tomobuchi-cho, Miyakojima-ku, Osaka 534, Japan and Research Laboratories, Torii & Co., Ltd.,[b] 14–3 Minamiyawata 3-chome, Ichikawa, Chiba 272, Japan.
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The wavelength dependency and the mechanism of photo-oxidation of ethyl 2-[4,5-bis(4-methoxyphenyl)thiazole-2-yl]pyrrol-1-ylacetate (KBT-3022) in solution were investigated. The wavelength contributing to the photo-degradation of KBT-3022 in a mixture of acetonitrile–H_2O (1 : 1) was found to be the near ultraviolet region, between 400 and 270 nm.

The photo-oxidation of KBT-3022 was enhanced by the presence of rose bengal, which generates singlet oxygen as a photosensitizer, and by the presence of D_2O in which the lifetime of singlet oxygen is about 10 times longer than in H_2O. On the other hand, photo-oxidation was inhibited by the presence of sodium azide, or 1,4-diazabicyclo[2.2.2]octane, both of which are known to be singlet oxygen quenchers. These results suggest that KBT-3022 acts by itself as a photosensitizer in its own photo-oxidation. Thus, the photo-degradation of *N*-methylpyrrole was enhanced by the presence of KBT-3022. It is concluded from these results that KBT-3022 reacts with singlet oxygen to form KBT-3022 *endo*-peroxide intermediate, and then undergoes hydrolysis to afford 5-hydroxylactam (D2).

Keywords KBT-3022; photo-oxidation; singlet oxygen; sensitizer; near ultraviolet light

Ethyl 2-[4,5-bis(4-methoxyphenyl)thiazole-2-yl]pyrrol-1-ylacetate (KBT-3022) is a new platelet aggregation inhibitor.[2,3)] In the previous paper, we reported the photo-stability of KBT-3022.[4)] Irradiation with ultraviolet (UV) light to KBT-3022 in a mixture of acetonitrile–H_2O (1 : 1) afforded substituted 5-hydroxylactam (D2) together with several minor products. However, the wavelength dependency and the mechanism of the photo-degradation of KBT-3022 has not been studied in detail.

Photo-labile drugs decompose under light of specific wavelengths. However, there are few reports relating to the wavelength contributing to photo-degradation of drugs. Matsuda *et al.* investigated the wavelength-dependence on the photo-degradation of indometacin[5)] and nifedipine[6)] by irradiating with light of a constant wavelength using monochromater. In this work, we investigated the wavelength dependency of the photo-degradation of KBT-3022 in solutions, using colored glass filters as recently reported.[7)]

In general, mechanisms by which photo-oxidation of organic compounds may occur are classified as follows.[8)] The Type I mechanism is a free radical chain process and is generally termed autooxidation. The Type II mechanism involves the excited singlet molecular oxygen and is termed oxygenation. It is well known that heterocyclic compounds such as pyrrole and furan undergo photo-oxidation by a Type II mechanism.[9)] Since KBT-3022 is a pyrrole derivative, it is assumed that KBT-3022 also undergoes photo-oxidation by a Type II mechanism. In this paper, we investigated the photo-oxidation of KBT-3022 to confirm the type of mechanism involved.

Experimental

Materials KBT-3022 was prepared in our laboratory and used without further purification. All other chemicals were of reagent grade, and water was taken from a Milli-QII water purification system (Millipore, Ltd.).

Irradiation Procedure Sample solutions for the irradiation procedure were prepared under protection from lights, using a mixture of acetonitrile–H_2O (1 : 1) or acetonitrile as a solvent. Sample solutions of KBT-3022 (100 μg/ml) contained 5 μM rose bengal as a singlet oxygen generator, or 10 mM sodium azide[10)] or 10 mM 1,4-diazabicyclo[2.2.2]-octane (DABCO)[11)] as singlet oxygen quenchers. The sample solution of *N*-methylpyrrole (100 μg/ml) contained 100 μM of KBT-3022 as a photosensitizer.

In the case of irradiation under a high pressure mercury lamp (300 W), an exposure test was performed with a merry-go-round type apparatus,[4,7,12)] as shown in our previous reports. The distance between the lamp and pyrex glass tube (15 ml) with a stopper containing the sample solution (15 ml) was approximately 120 mm. The sample solution was exposed to light transmitted through a series of colored glass filters (Toshiba Garasu Kogyo Co., Ltd.) attached to the apparatus of the light source. The intensity of irradiation, to which the sample solutions were exposed, was measured as UV light (300—400 nm) energy per cm^2 using a UV-intensity meter, model UVX, with a UVX-36 Sensor (UVP, Inc.).

When fluorescent lamps (20 W × 6, white light, Type FLR20S W/M, National Co., Ltd.) or chemical lamps (20 W × 6, Type FLR20S BL/M, Toshiba Electric Co., Ltd.) were used as a light source, a test box[4,12)] to which the colored glass filters were attached was placed in the photo-stability test instrument (Light-Tron, model LT-120, Nagano Science Co., Ltd.). The illumination of the fluorescent lamp was adjusted to 2500 lx, and the irradiation intensity of the chemical lamp was adjusted to 1000 μW/cm^2.

Assay of KBT-3022 and *N*-Methylpyrrole The determination of intact KBT-3022 was performed by high performance liquid chromatography (HPLC) as previously described.[4)] For the determination of intact *N*-methylpyrrole, 10 μl of the sample solution was injected into the HPLC system. The HPLC system was the same as that used for the assay of KBT-3022; except for the Inertsil ODS2 5-250 column (4.6 i.d. × 250 mm, particle size 5 μm, GL Sciences Inc.) and the wavelength used for detection (215 nm).

Results and Discussion

Wavelength Affecting Photo-Degradation of KBT-3022 We previously proposed a simple new method for the quantitative photo-stability estimation of a drug.[7)] In this

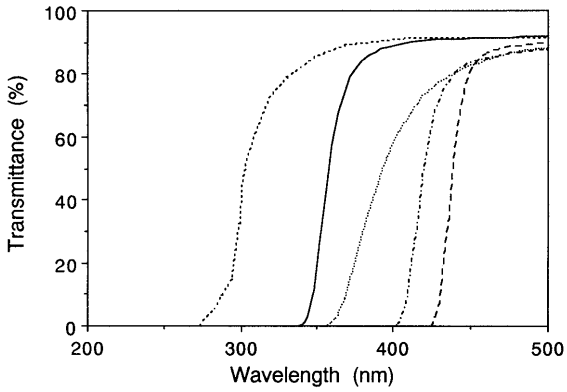

Fig. 1. Spectral Transmittance of Colored Glass Filters and Pyrex Glass Tube

Colored glass filter: ——, UV-35; ------, L-39S; —·—, L-42; ——, Y-44; – – –, Pyrex glass tube.

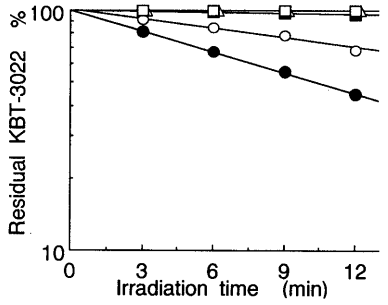

Fig. 2. Effect of Wavelength on the Photo-Degradation of KBT-3022 in the Mixture of Acetonitrile–H_2O (1 : 1) at Room Temperature

Colored glass filter: ○, UV-35; ■, L-39S; □, L-42; △, Y-44; ●, control (without glass filters). Initial concentration of KBT-3022 was 100 μg/ml.

report, we have determined the range of the wavelength contributing to photo-degradation of KBT-3022 using this new method. In our preliminary work, KBT-3022 was found not to absorb visible light, but near UV light. Also, KBT-3022 in the mixture of acetonitrile–H_2O shows an absorbance maximum at approximately 345 nm which belongs to the near UV region, probably contributing to the photo-degradation. Therefore, the photostability of KBT-3022 in the mixture of acetonitrile–H_2O was investigated using colored glass filters. UV-35, L-39S, L-42 and Y-44 colored glass filters, which cut off the light of the wavelength region below approximately 340, 355, 400 and 425 nm, respectively, were selected, as shown in Fig. 1. The irradiation intensity, when UV-35, L-39S color glass filters or no filters were attached, was 20, 420 and 700 μW/cm^2, respectively. In the case of L-42 and Y-44, each value was 0 μW/cm^2.

The photo-degradation of KBT-3022 under the light of a high pressure mercury lamp, which has strong line spectra in both the visible and near UV regions, was investigated. KBT-3022 decomposed under light transmitted through UV-35 or L-39S, as shown in Fig. 2. The photo-degradation rate became faster as the transmittance of light in the near UV region increased. On the other hand, KBT-3022 was quite stable when exposed to light when L-42 or Y-44 was attached to the apparatus of the light source. From these results, it is suggested that

Table I. Photo-Degradation of KBT-3022 under Light from Fluorescent Lamps and Chemical Lamps Transmitted through Various Colored Glass Filters

Light source	Residual KBT-3022 (%)				
	Colored glass filter				
	UV-35	L-39S	L-42	Y-44	Control[a]
Fluorescent lamps[b]	86.7	95.9	99.7	99.9	80.8
Chemical lamps[c]	66.9	94.3	99.6	99.9	36.3

Each value represents the mean of the residual percentage of KBT-3022 ($n=2$).
a) Without glass filters. *b*) Apparent integrated illumination was 50000 lx·h.
c) Apparent integrated irradiation intensity was 2000 μW·h/cm^2.

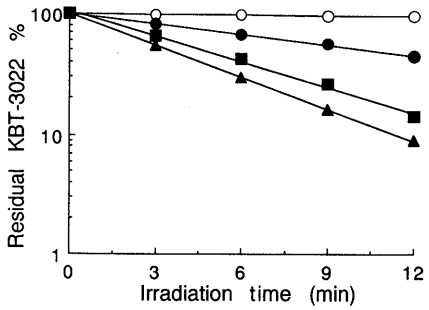

Fig. 3. Effect of Various Additives on the Photo-Degradation of KBT-3022 in the Mixture of Acetonitrile–H_2O (1 : 1) at Room Temperature

Additive: ▲, 5 μM rose bengal; ■, D_2O–CH_3CN; ○, 10 mM azide; ●, control (without additives). Initial concentration of KBT-3022 was 100 μg/ml.

the irradiation intensity of the near UV region light, in which the absorption maximum of KBT-3022 exists, relates to the photo-degradation of KBT-3022.

Furthermore, the photo-degradation of KBT-3022 under the light of fluorescent lamps or chemical lamps was investigated. The light of fluorescent lamps irradiated for 20 h, and that of chemical lamps irradiated for 2 h. As given in Table I, the visible light transmitted through L-42 and Y-44 of the two light sources had no effect on the photo-degradation of KBT-3022. But when UV-35 and L-39S were used, the photo-degradation of KBT-3022 occurred as the result of near UV light from both light sources. Pyrex glass tubes used in the experiment cut off light from the wavelength region below approximately 270 nm,[7] as shown in Fig. 1.

These results suggest that KBT-3022, in the mixture of acetonitrile–H_2O, is degraded by near UV light between 400 and 270 nm.

Photo-Degradation Scheme of KBT-3022 Heterocyclic compounds, such as pyrrole and furan, afford *endo*-peroxide intermediates which are formed by the 1,4-addition of singlet oxygen when exposed to light in the presence of a sensitizer such as rose bengal, eosin or methylene blue.[9,13] Pyrrole *endo*-peroxide intermediate affords 5-hydroxylactam by hydrolysis or rearrangement.[9] Since KBT-3022 afforded substituted 5-hydroxylactam (D2) by exposure to light, we predicted that the photo-oxidation of KBT-3022 occurred by a Type II mechanism.

Rose bengal was used as a singlet oxygen generating

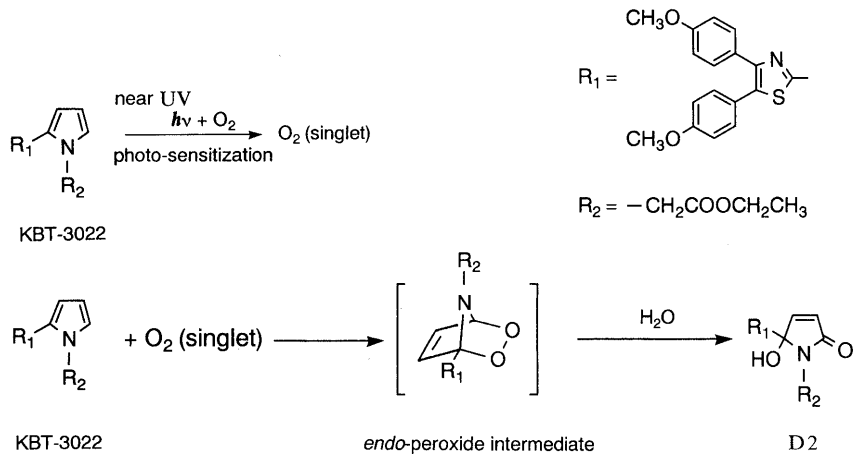

Chart 1. Proposed Mechanism of the Photo-Degradation of KBT-3022 in the Mixture of Acetonitrile–H_2O (1 : 1)

sensitizer. For an exposure test, light from a high pressure mercury lamp without a glass filter was used. The photo-oxidation of KBT-3022 was enhanced by the presence of rose bengal (Fig. 3). Additionally, the photo-oxidation of KBT-3022 occurred in the presence of rose bengal, despite the use of an L-42 colored glass filter which cut off light below approximately 400 nm.

In general, the lifetime of singlet oxygen is dependent on the nature of the solvent. It is well known that there is a deuterium effect on the lifetime of singlet oxygen. The lifetime of singlet oxygen is about 10 times longer in D_2O than in H_2O.[14] When a sample solution of KBT-3022 in a mixture of acetonitrile–D_2O (1 : 1) was exposed to light, the photo-oxidation was accelerated, compared to that in the mixture of acetonitrile–H_2O (Fig. 3).

Furthermore, it is well known that sodium azide[10] and DABCO[11] efficiently quench singlet oxygen. When sodium azide was added to a sample solution of KBT-3022, the photo-oxidation was inhibited, and the amount of the degradation product (D2) was below the detection limit. In acetonitrile, the photo-oxidation of KBT-3022 was also depressed by the presence of DABCO, and was enhanced by the presence of rose bengal.

These results suggest that the photo-oxidation of KBT-3022 proceeds through a Type II mechanism. That is to say, KBT-3022 acts as a sensitizer for its own oxidation.

Therefore, KBT-3022 may act as a photosensitizer in photo-oxidation as the result of a Type II mechanism, such as that of *N*-methylpyrrole.[9] Thus, the photo-degradation of *N*-methylpyrrole in a mixture of acetonitrile–H_2O under the light of a high pressure mercury lamp with a UV-35 colored glass filter was investigated. The photo-degradation of *N*-methylpyrrole was enhanced by the presence of KBT-3022.

It is concluded from these results that KBT-3022 reacts with singlet oxygen to form a KBT-3022 *endo*-peroxide intermediate, and then undergoes hydrolysis to afford D2, as presented in Chart 1.

References and Notes

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