Photodecarboxylation of DX-9065a, a New Factor Xa Inhibitor, in Aqueous Solution

Yukinori KAWAI* and Kyuichi MATSUBAYASHI

Drug Metabolism and Analytical Chemistry Research Laboratory, Daiichi Pharmaceutical Co., Ltd., 16–13, Kita-Kasai 1-chome Edogawa-ku, Tokyo 134, Japan. Received August 12, 1997; accepted October 13, 1997

The photodegradation reaction of (2*S*)-2-[4-[[(3*S*)-1-acetimidoyl-3-pyrrolidinyl]oxy]phenyl]-3-(7-amidino-2-naphthyl)propanoic acid hydrochloride pentahydrate (DX-9065a), a new factor Xa inhibitor, was investigated in aqueous solution at various pH values (1.1—8.0) at 25 °C. The photodegradation of DX-9065a followed apparent first-order kinetics under artificial sunlight in a fairly good manner. The photodegradation rates of DX-9065a in a neutral solution were higher than those in acidic solution. The log *k*-pH profile indicated that the photodegradation rate of DX-9065a was related to the dissociation of the carboxyl group and suggested that the main photo-labile species was the carboxylate form. Further kinetic study showed that the carboxylate form of DX-9065a underwent decarboxylation quantitatively to produce D41-1077, the photodecarboxylation product, on irradiation, while the carboxylic acid form of DX-9065a did not perform any photodecarboxylation. Moreover, the photodecarboxylation mechanism was discussed in relation to the kinetics and photodegradation pathway.

Key words photodegradation; photodecarboxylation; kinetics; DX-9065a

The stability of a drug is generally an important factor in planning the transport, storage and manufacturing process of a preparation. The stability data thus forms an essential part of the documents in a new drug application. Degradation kinetics of drugs in aqueous solution have therefore been discussed by many researchers,[1] and the effects of the pH, temperature, dissolved oxygen, additives and structural properties have been investigated in detail as potential factors affecting drug stability.

Light irradiation is also a significant factor affecting stability, so that photostability data is one important part of the stability data. The effects of storage conditions, such as wavelength of light[2] and initial drug concentration,[3] on the photostability have been investigated extensively for many drugs. In the case of ionizable compounds such as nifedipine[4] and ciprofloxacin,[5] the effect of pH is one of the most significant factors, because there are large differences between the photostability of the ionic and nonionic forms. This makes it necessary to examine the relationships between the dissociation of functional groups and photodegradation kinetics, and to study the photostability of each species, the dissociated and undissociated forms.

DX-9065a, (2*S*)-2-[4-[[(3*S*)-1-acetimidoyl-3-pyrrolidinyl]oxy]phenyl]-3-(7-amidino-2-naphthyl)propanoic acid hydrochloride pentahydrate, is a new factor Xa inhibitor, which has recently been subjected to clinical trial. The compound has a carboxyl group, an acetimidoyl group and an amidino group in its molecule and is also ionizable (Fig. 1). Ono *et al.* studied geometrical isomerism of DX-9065a at various pH in aqueous solution.[6] However, the stability of this compound has not yet been investigated. This study describes the photostability of DX-9065a in aqueous solution in detail. We also investigated the kinetics, pathway and mechanism of the photodegradation reaction.

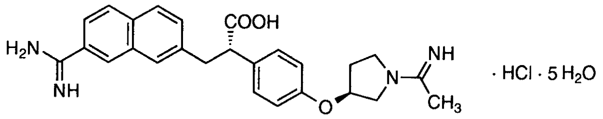

Fig. 1. Chemical Structure of DX-9065a

Results and Discussion

Rate Constants Aqueous buffer solutions of pH ranging from 1.1 to 8.0 were used as the solvent in this experiment. Buffer solutions of pH above 8.0 were not used in order to avoid rapid hydrolysis of the acetimidoyl and amidino groups of DX-9065a, because these functional groups are easily hydrolyzed in alkaline solution. Figure 2 shows that the decrease in concentration of DX-9065a with integral illumination intensity followed apparent first-order kinetics at pH range 1.1 to 8.0. The first-order plots exhibited good linear relationships with correlation coefficients of 0.96 or higher at all pH examined. The apparent first-order rate constants (k_{obs}) were obtained from slopes of the plots. When kept in the dark, the solutions of DX-9065a were stable throughout the experimental period.

Dissociation Constants of DX-9065a The dissociation constant (pK_a) of the carboxyl group of DX-9065a was 3.4, and pK_a values of amidino and acetimidoyl groups of DX-9065a were above 11.

log *k*–pH Profiles for Photodegradation of DX-9065a The log *k*–pH profile for the photodegradation of DX-

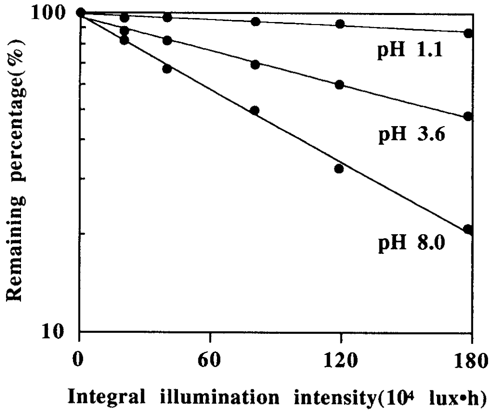

Fig. 2. Photodegradation Kinetics of DX-9065a in Aqueous Solution at Various pH

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* To whom correspondence should be addressed.

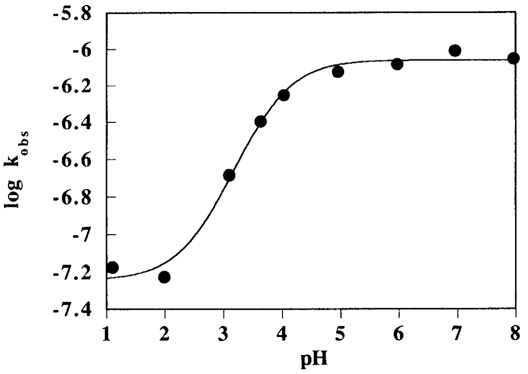


Fig. 3. log k–pH Profile for the Photodegradation of DX-9065a
Points: experimental, lines: theoretical curve.

9065a is shown in Fig. 3. The photodegradation rates in neutral solution were higher than those observed in acidic solution of pH below 2. There was an inflection point near pH 3. Since the dissociation constant of the carboxyl group of DX-9065a is pK_a 3.4, the carboxylic acid form is predominant below pH 2, while the carboxylate form is predominant above pH 4. The amidino group and acetimidoyl group are completely protonated in the range of pH 1 to 8 and their ionic conditions do not change. Therefore, the log$k$–pH profile shows that there is a large difference between the photostability of the carboxylic acid form and the carboxylate form. This means that the photodegradation rates are affected by dissociation of the carboxyl group of DX-9065a. Based on the relationship between the photodegradation rate and dissociation of the carboxyl group of this inhibitor, the following equations can be derived:

$$C_{AT}=C_{HA}+C_{A} \tag{1}$$
$$K_a=C_A\cdot a_H/C_{HA} \tag{2}$$
$$-dC_{AT}/dI=k_1\cdot C_{HA}+k_2\cdot C_A \tag{3}$$

where C_{AT}, C_{HA} and C_A are the concentration of the total, the carboxylic acid form and carboxylate form of DX-9065a, respectively; k_1 and k_2 are the first-order rate constants for the photodegradation of carboxylic acid form and carboxylate form of DX-9065a, respectively; a_H is the hydrogen-ion activity; K_a is the dissociation constant of the carboxyl group; and I is the integral illumination intensity.

Combining Eqs. 1, 2 and 3 gives Eq. 4 and Eq. 5:

$$-dC_{AT}/dI=k_{obs}\cdot C_{AT} \tag{4}$$
$$k_{obs}=(k_1\cdot a_H+k_2\cdot K_a)/(K_a+a_H) \tag{5}$$

The values of k_1, k_2 and K_a were calculated from the data shown in Fig. 3 using Eq. 5 by non-linear least squares fit method. The results estimated from the best fits of the log$k$–pH profile are as follows:

$$\log k_1=-7.24$$
$$\log k_2=-6.06$$
$$pK_a=3.8$$

In Fig. 3, the line represents the theoretical curve calculated from Eq. 5. The reasonable degree of agreement between the theoretical and experimental values indicates that Eq. 5 adequately describes the kinetics of the photodegradation reaction. Furthermore, the pK_a value (3.8) derived by the kinetic study agreed comparatively well with the value obtained from the potentiometric study (3.4). Thus, these results indicate that the carboxylate form of DX-9065a is photo-labile, and the photodegradation rate of the carboxylate form is about 15 times that of the carboxylic acid form. According to Eq. 5, the photostability of DX-9065a in aqueous solution can be predicted at any pH.

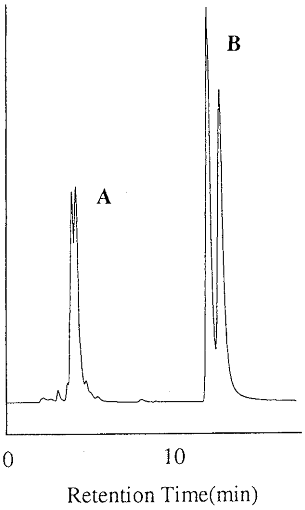


Fig. 4. HPLC Chromatogram of DX-9065a and Its Degradation Product in pH 7.0 Buffer Solution of DX-9065a Irradiated at 180 × 10^4 lux·h of Artificial Sunlight
(A), DX-9065a; (B), D41-1077.

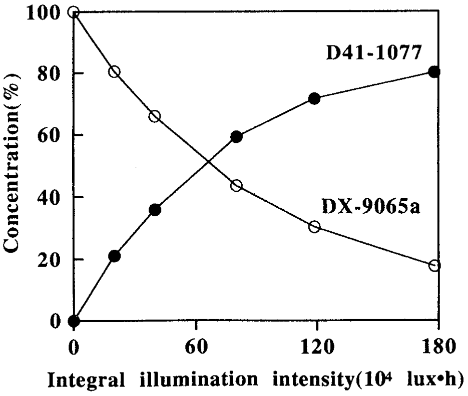


Fig. 5. Relative Molar Concentrations of DX-9065a and D41-1077 in pH 7.0 Buffer Solution of DX-9065a Irradiated by Artificial Sunlight at 25 °C

Photodegradation Product in Aqueous Solution D41-1077, which was generated by decarboxylation of DX-9065a as described below, was detected as one major photodegradation product of DX-9065a in aqueous solution by HPLC (Fig. 4). Both DX-9065a (peak A) and D41-1077 (peak B) were observed as two peaks on the HPLC chromatogram, respectively. This is due to the fact that two geometrical isomers, *E* and *Z*, are possible, because DX-9065a and D41-1077 each has an acetimidoyl group in its molecules.[6] The time course of the photodegradation of DX-9065a in pH 7.0 solution is shown in Fig. 5. The concentration of DX-9065a decreased with integral illumination intensity. In concert with this process, the concentration of D41-1077 increased, and DX-9065a

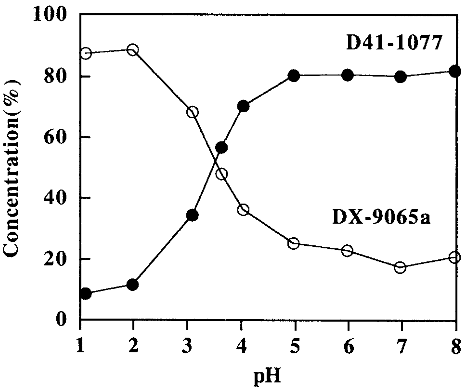
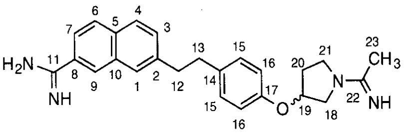


Fig. 6. Relative Molar Concentrations of DX-9065a and D41-1077 in Various pH Solutions of DX-9065a Irradiated at 180 × 10^4 lux·h of Artificial Sunlight

Table 1. Analytical Results of D41-1077

^{1}H-NMR (DMSO-d_6)	2.19—2.22 (m, 2H, 20-H), 2.26, 2.30 (both s, 3H, 23-H), 2.95 (t, 2H, $J=8$ Hz, 13-H), 3.08 (t, 2H, $J=8$ Hz, 12-H), 3.57—3.96 (m, 4H, 18-H, 21-H), 5.12, 5.18 (both s, 1H, 19-H), 6.88, 6.89 (both d, 2H, $J=7$ Hz, 16-H), 7.20 (d, 2H, $J=7$ Hz, 15-H), 7.63 (d, 1H, $J=8$ Hz, 3-H), 7.78 (d, 1H, $J=9$ Hz, 7-H), 7.84 (s, 1H, 1-H), 7.98 (d, 1H, $J=8$ Hz, 4-H), 8.08 (d, 1H, $J=9$ Hz, 6-H), 8.41 (s, 1H, 9-H), 9.32—9.91 (brs, 4H, 11-NH$_2$, 11-NH, 22-NH)
^{13}C-NMR (DMSO-d_6)	18.40, 18.44 (23-C), 29.90, 30.04 (20-C), 35.65 (13-C), 37.04 (12-C), 46.38, 47.25 (21-C), 53.50, 54.35 (18-C), 75.17 (19-C), 115.43, 115.47 (16-C), 122.78 (7-C), 125.98 (8-C), 127.29 (1-C), 127.60 (4-C), 128.26 (6-C). 128.46 (9-C), 129.52 (15-C), 130.27 (3-C), 131.81 (10-C), 133.42 (5-C), 133.99, 134.06 (14-C), 140.82 (2-C), 154.53, 154.65 (17-C), 165.76 (22-C), 165.91 (11-C)
FAB-MS	m/z 401 [M+H]$^+$

Carbon position was indicated as follows:



yielded D41-1077 almost quantitatively. Figure 6 shows the results of the residual amount of DX-9065a and the amount of D41-1077 formed in the various pH solutions of DX-9065a irradiated 180 × 10^4 lux·h by artificial sunlight. The total concentration of each was approximately 100% at any pH, and the unknown photodegradation products detected by HPLC were in very small quantities. These findings indicated that the main photodegradation product of DX-9065a was D41-1077.

The chemical structure of D41-1077 was elucidated by comparing the ^{1}H- and ^{13}C-NMR spectra, and the MS of D41-1077 with those of DX-9065a. The analytical results of D41-1077 are summarized in Table 1. The NMR spectral patterns of D41-1077 were similar to those of DX-9065a, and some NMR signals of D41-1077 were observed as twin signals each attributable to the two geometrical isomers. Methylene proton and carbon signals of the benzylic position were newly observed at 2.9 ppm and 35.6 ppm, respectively. Correspondingly, methine proton and carbon signals at the benzylic position of DX-9065a disappeared in the NMR spectra of D41-1077. Carbon signal of carboxyl group of DX-9065a also disappeared in the ^{13}C-NMR spectrum of D41-1077. The quasimolecular ion was observed at m/z 401 in FAB-MS. This molecular weight is 44 mass units less than that of DX-9065a. These findings support the chemical structure of D41-1077, a decarboxylated derivative of DX-9065a, as shown in the footnote of Table 1.

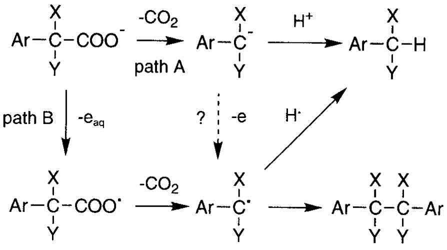


Chart 1. Proposed Mechanism of Photodecarboxylation Based on Isobe *et al.*[7)]

Mechanism of Photodegradation On irradiation of the carboxylate form of DX-9065a in neutral solution, a carboxyl group at the benzylic position was quantitatively removed to give the D41-1077. On irradiation in 6 N hydrochloric acid, however, the carboxylic acid form of DX-9065a did not undergo decarboxylation and nearly 100% was recovered unchanged.

It has been reported that arylacetic acid, when the carboxyl group is attached to a secondary or tertiary carbon, is decarboxylated in considerable yield on irradiation of its aqueous alkaline solution, while the corresponding free acids are not decarboxylated.[7)] This phenomenon is due to the fact that carboxylate is more labile than free acid for photodecarboxylation. The formation of D41-1077 from DX-9065a, which had a carboxyl group at the benzylic position, was believed to fit this reaction category. In this category, two mechanisms are known (Chart 1)[7)]: one is direct breakdown of the excited carboxylate ion to the benzyl anion (path A),[8)] and the other is ejection of a solvated electron from the carboxylate ion to give the carboxylate radical, which breaks down into carbon dioxide and benzyl radical (path B).[9)] Spin trapping reagents are generally used to distinguish path B from path A, and the obtained spin adducts are investigated. An attempt was made to obtain some spin adducts in the photodecarboxylation of DX-9065a using 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), which has been widely used as a spin trapping reagent.[10)] No significant ESR signal of spin adduct was observed in pH 7.0 buffer solution of DX-9065a containing DMPO on irradiation. Nor was decarboxylative dimerization of benzyl radical observed by HPLC analysis. Although no direct evidence of the mechanism such as trapping intermediate was available from our results, benzyl anion was thought most appropriate as the photodecarboxylation intermediate of DX-9065a.

Consequently, from the results of the log k–pH profile, the structure of the photodegradation product identified in solution, and lack of radicals in the reaction, the mechanism of the photodegradation of DX-9065a is proposed as shown in Chart 2. Namely, the carboxylic

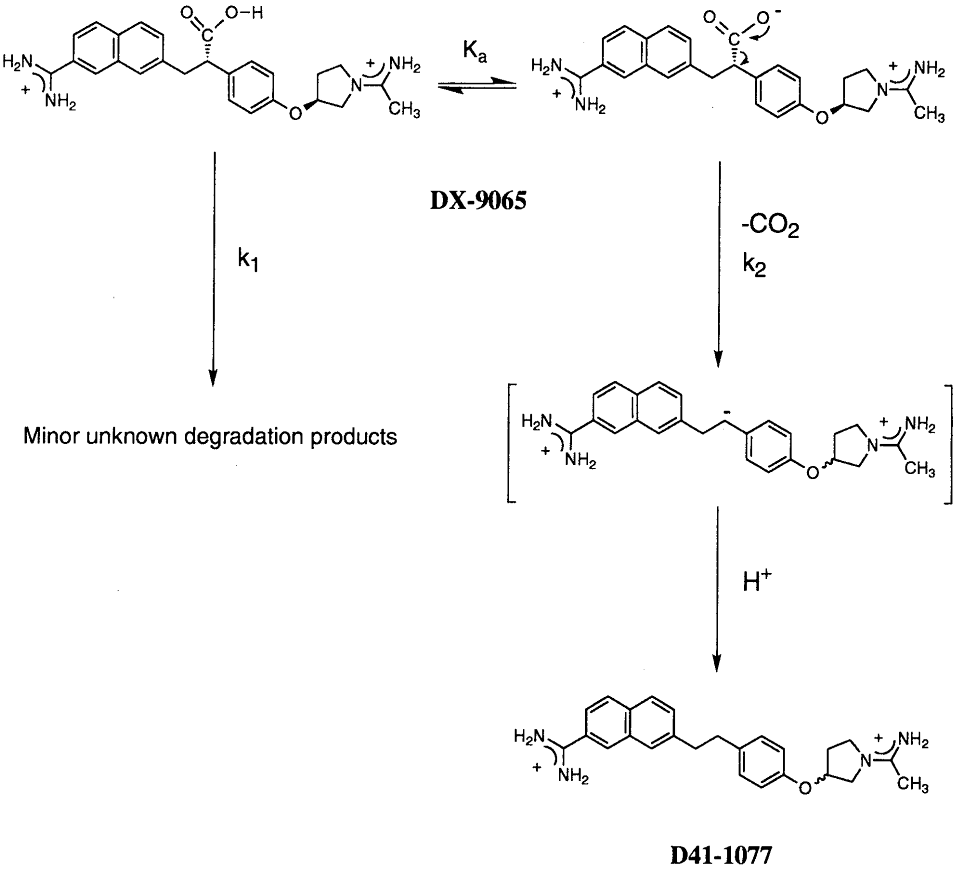


Chart 2. Proposed Mechanism of Photodegradation of DX-9065a

acid form of DX-9065a is believed to decompose to D41-1077 *via* benzyl anion, which is protonated by solvent.

The results of our study could provide a useful key to improve the photostability of products of DX-9065a. For instance, to improve the photostability of injectable DX-9065a requires avoiding the formation of its carboxylate ion in aqueous solution by such means as preparing it in acidic solution.

Experimental

Materials DX-9065a was synthesized at the Production Technology Research Laboratory of Daiichi Pharmaceutical Co., Ltd. (Tokyo). All solvents and reagents used in this experiment were of analytical and reagent grades. The water used was purified with a Milli-Q system (Millipore Co., Ltd., MA, U.S.A.).

HPLC Analysis of DX-9065a and D41-1077 HPLC analysis of DX-9065a and D41-1077 was carried out using the Shimadzu LC-10A system (Shimadzu Co., Kyoto). Samples of 20 μl were injected onto a reversed phase column (TSK gel-ODS 80TM column, 4.6 mm i.d. × 15 cm, Tosoh, Tokyo). DX-9065a and D41-1077 were monitored by UV absorption at 242 nm. The mobile phase consisted of a mixture (75 : 25) of 0.05 M Sϕrensen buffer and acetonitrile, and flow rate was 0.7 ml/min (0—10 min) and 1.0 ml/min (10—20 min) at room temperature.

Kinetic Study The photodegradation kinetics of DX-9065a was studied in aqueous solutions at various pHs (1.1—8.0). Britton–Robinson buffer solution of pH 2.0—8.0 and 0.1 N hydrochloric acid (pH 1.1) were used as solvents. DX-9065a was dissolved in these solutions and the resulting solutions (1.0 mg/ml) were used as samples. Each sample solution (2 ml) was poured into a glass container(internal diameter 18 mm), which was covered with clear wrapping film made of polyvinylidene chloride (Kureha Kagaku Kogyo Co., Ltd., Tokyo). Then, the exposure test was performed at 25 °C by irradiating these sample solutions with artificial sunlight (FLR40S W-E/M, Toshiba Electric Co., Ltd., Tokyo). Artificial sunlight used as a light source radiates light of a wavelength from *ca.* 300 nm to *ca.* 700 nm, and *ca.* 8000 lux of illumination intensity. Illumination intensity was measured by an illumination meter IM-3 (Topcon Co., Tokyo). At appropriate time intervals, a portion (0.2 ml) of the sample solution was withdrawn from the glass container, mixed with 1.8 ml of the mobile phase, and assayed by HPLC.

Isolation of D41-1077 DX-9065a (100 mg) was dissolved in water so that the total volume was 100 ml. This sample was exposed to artificial sunlight for 3 d. After irradiation, the solution was subjected to preparative HPLC to obtain a photodegradation product, D41-1077. Preparative HPLC was performed in the following manner using a Hitachi HPLC system (type 655). The HPLC column was TSK gel ODS-80TM, 21.5 mm i.d. × 30 cm column. D41-1077 was monitored by absorption at 250 nm. The mobile phase consisted of a mixture (70 : 30) of 1% acetic acid and acetonitrile, and flow rate was 5.0 ml/min at room temperature. ^{1}H-NMR spectra and ^{13}C-NMR spectra of isolated D41-1077 in dimethyl sulfoxide-d_6 (DMSO-d_6) were measured at 25 °C with a JNM-GSX500 NMR spectrometer (JEOL, Ltd., Tokyo). Tetramethylsilane was used as an internal standard. Fast atom bombardment mass spectrum (FAB-MS) of D41-1077 was measured using a JMS-HX110 mass spectrometer (JEOL, Ltd.).

Determination of Dissociation Constants of DX-9065a The dissociation constant of DX-9065a was determined by pH titration method. The pH titration of solution containing DX-9065a (12.5 mM) with 1.0 N NaOH was carried out under nitrogen atmosphere at 20 °C. Measurements of pH were performed with an automatic titratorGT-05 (Mitsubishi Kasei Co.) equipped with a GTPH10 glass electrode and a GTPH50 reference electrode. The pK_a values were calculated using the computer program PKAS.[11]

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