λ-Radiolysis of Mitomycin C Derivatives

Tokuyuki Kuroda*, Koji Hisamura and Nobuhiro Nakamizo

Pharmaceutical Research Laboratories,
Kyowa Hakko Kogyo Co. Ltd.,
Nagaizumi, Sunto-gun, Shizuoka 411, Japan

Yoshio Otsuji

Department of Applied Chemistry, College of
Engineering, University of Osaka Prefecture,
Sakai, Osaka 593, Japan

Received April 23, 1993

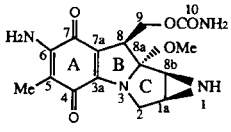
The λ-Radiolysis reactions of mitomycin C (**1**) and its derivatives were studied in the hope of developing a radiation-induced drug (RID). The λ-radiolysis reactions were carried out in aqueous solutions under the condition where hydrated electron (e^-_{aq}) was generated as a principal reactive species. The competitive λ-radiolysis studies revealed that the rate constants for the reactions of **1** with e^-_{aq} at room temperature was 3.6 x 10^{10} dm^3 $mol^{-1}s^{-1}$. Among mitomycin C derivatives, the 5*H*-6-alkoxyimino derivatives **11** and **12**, and compound **13** in which ring A of **1** has the 4-hydroxy-6-hydroxyimino structure cleaved to give **1**. The mechanic aspect of these λ-radiolysis reactions is discussed.

J. Heterocyclic Chem., **31**, 53 (1994).

Introduction.

Previously, we have proposed the concept of "Radiation-Induced Drug (RID)" as a new type of drug, particularly for cancer therapy [1]. This drug is a sort of prodrug in which an active drug is protected in some ways by chemical modification, but the active drug can be regenerated upon γ-irradiation. On the basis of this concept, we have studied γ-radiolyses of various derivatives of well-specified antitumor agents such as 5-fluorouracil (5-FU) [1], 5-fluorouridine (5-FUR) [2,3] and 5-fluorodeoxyuridine (5-FUdR) [2]. In these studies, we have found that some of the derivatives, particularly the compounds having sulfur-containing substituents at the nitrogen of heterocyclic moiety or at the oxygen of sugar moiety, release the active drugs with high G values upon γ-irradiation of their aqueous solutions [2,3].

Mitomycin C (**1**) has a strong cytotoxic activity and is one of the widely used antitumor agents. Therefore, we have sought substances to release **1** upon γ-irradiation. We now report the γ-radiolysis of various types of mytomycin C derivatives that have been synthesized in a preceeding paper [4]. In this paper the reactivity features of **1** in the γ-radiolysis in aqueous solutions are also described.

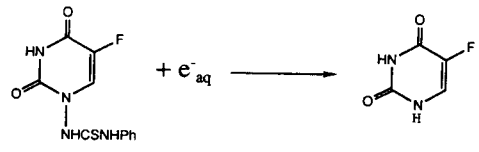


1

Results and Discussion.

γ-Radiolysis of **1**.

Since **1** contains the *p*-quinone moiety in ring A of the molecule, this compound seems to be sensitive to an hydrated electron (e^-_{aq}). Hence, we tried to estimate the ability of **1** for capturing e^-_{aq} by utilizing a competitive γ-radiolysis method. As reference reactions of the competitive γ-radiolysis reactions, we chose the reaction of 5-fluoro-1-(phenylthioureido)uracil (**2**) with e^{-1}_{aq} to yield 5-FU, (**3**), whose rate constant is already known [5].



2 **3**

The kinetic expression for the competitive γ-radiolysis reactions can be written by Equations 1-5. In these equations, k_1 and k_2 denote the rate constants for the reactions shown in Equations 1 and 2, P_1 is the primary products obtained by the reaction of **1** with e^-_{aq}. The G(X)s are the G values for the formation of the species shown in parentheses.

$$\mathbf{1} + e^-_{aq} \xrightarrow{k_1} P_1 \qquad (1)$$

$$\mathbf{2} + e^-_{aq} \xrightarrow{k_2} \mathbf{3} \qquad (2)$$

$$G(P_1) = \frac{k_2\,[1]}{k_1\,[1] + k_2\,[2]} \cdot G(e^-_{aq}) \qquad (3)$$

$$G(\mathbf{3}) = \frac{k_2\,[2]}{k_1\,[1] + k_2\,[2]} \cdot G(e^-_{aq}) \qquad (4)$$

$$G(e^-_{aq}) = G(P1) + G(\mathbf{3}) \qquad (5)$$

In deriving these equations, we neglected the detailed mechanisms for the reactions. Indeed, there is an indication that **3** is formed *via* the radical anion of **2** [1]. Nevertheless, we assumed that these equations represent the re-

activity feature of the γ-radiolysis reaction under consideration in a fairly good approximation. From these equations, Equation 6 can be derived. The rate constant k_1 expresses the reactivity of **1** toward e^-_{aq}. The value of k_1 can be estimated by applying Equation 6 without knowing the detailed structure of P_1.

$$\frac{1}{G(3)} = \frac{1}{G(e^-_{aq})} + \frac{1}{G(e^-_{aq})} \cdot \frac{k_1[1]}{k_2[2]} \quad (6)$$

The competitive γ-radiolysis with respect to e^-_{aq} was carried out by irradiating deaerated aqueous 1% (v/v) methanol solutions of **1** containing varing amounts of **2** at room temperature with γ-rays of 50 Gy from a ^{137}Cs source at a dose rate of 3.15 Gy/minute. The G(**3**) values were determined by measuring the amounts of **3** produced by the γ-radiolysis. We found that although **1** captures rapidly e^-_{aq}, the compound itself is chemically stable under the reaction conditions. In fact, most of **1** was recovered after aerating the reaction mixture.

It is known that the γ-radiolyses under the above condition produce e^-_{aq} as a principal reactive species and the rate constant k_2 under the same condition is 1×10^{10} dm^3 mol^{-1} s^{-1} [5]. Figure 1 shows the plots of the $G(3)^{-1}$ values against the concentration ratios [**1**] / [**2**]. The plot gave the straight line. This result verifies the validity of Equation 6. The rate constant k_1 was then calculated by dividing the slope of the straight line by the intercept and also by using the k_2 value cited above. The calculated rate constant is given in Figure 1: $k_1 = 3.6 \times 10^{10}$ dm^3 mol^{-1} s^{-1}. This result suggests that the ability of **1** for capturing e^-_{aq} is about 3-4 times greater than that of **2**. It is noteworthy that **1** reacts efficiently with e^-_{aq}. It is also worthy to note that the $G(e^-_{aq})$ value (2.2) calculated from the intercept of Figure 1 is in fairly good agreement with the experimentally determined $G(e^-_{aq})$ value (2.6) [6] under the similar conditions. This result indicates that the assumptions made on deriving Equation 6 is reasonable and the result obtained here is fairly reliable.

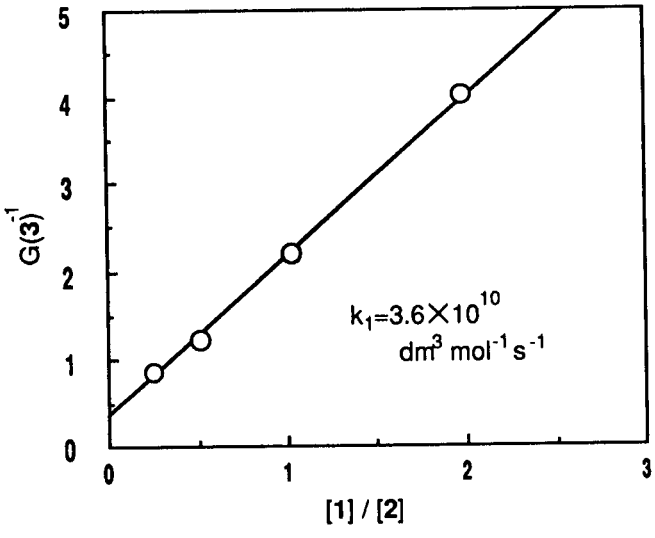
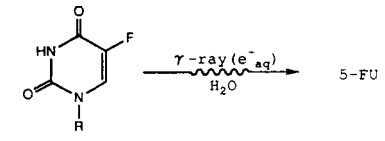


Figure 1. Plots of $G(3)^{-1}$ value against the concentration ratios [**1**] / [**2**].

γ-Radiolysis of Mitomycin Derivatives.

Previously, we found that the 5-FU derivatives **4** and **5** efficiently produce 5-FU upon γ-irradiation in aqueous solutions, particularly *via* the reaction with e^-_{aq} (Scheme 1).

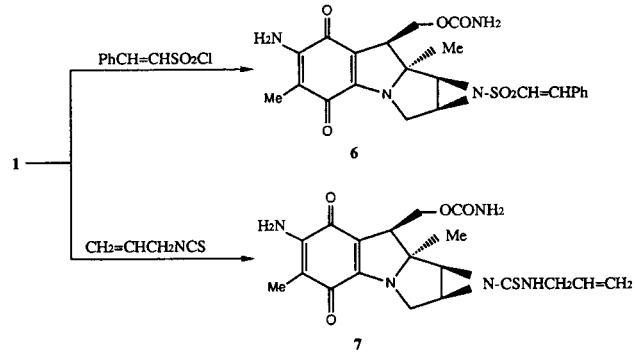
Scheme 1



4 : R = PhCH=CHSO$_2^-$

5: R = PhCH$_2$NHCSNH-

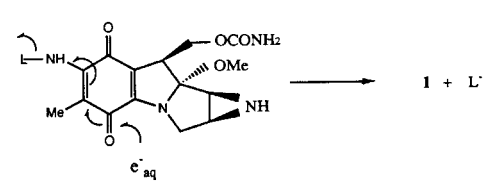
Scheme 2



Therefore, we first examined the γ-radiolysis of mitomycin C derivatives **6** and **7** that are prepared from **1** (Scheme 2).

However, when the γ-radiolysis of **6** and **7** was carried out in aqueous 1% (v/v) methanol, **1** could not be detected at all. This observation suggests that in the γ-radiolysis of **6** and **7**, e^-_{aq} attacks the quinone moiety at a much faster rate than the sulfonyl and thioureido moieties. Since the quinone moiety of **1** is especially reactive toward e^-_{aq}, we thought that compounds of type **8** may be cleaved by the attack of e^-_{aq} to give **1**, if L is a good leaving group

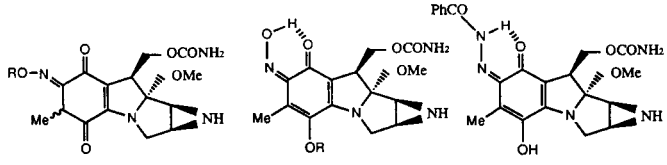
Scheme 3



8

(Scheme 3). Furthermore, it is known that N-N and N-O bonds are relatively weak and susceptible to reductive cleavage: the bond energies of N-N and N-O bonds are 39 and 53 Kcal mol^{-1}, respectively [7].

With these considerations in mind, we studied the γ-radiolysis of compounds **9-13**, which we have prepared in the preceeding paper [4]. The γ-radiolysis was carried out in aqueous solutions under the conditions where e^-_{aq} is generated as a principal reactive species. The results are given in Table 1.



9 : R = Me
10 : R = i-Bu
11 : R = H
12 : R = Me
13

Table 1

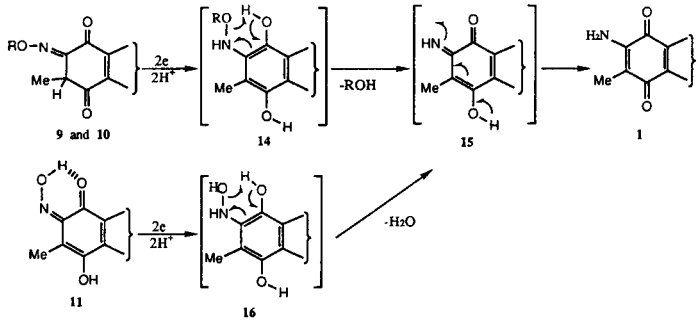
γ-Radiolyses of Mitomycin C Derivatives

Compound	G(1) value
9	0.15
10	0.53
11	0.20
12	none [a]
13	none

[a] None indicates that **1** was not detected.

Compounds **9**, **10**, and **11** gave **1** by the reaction with e^-_{aq}. However, compounds **12** and **13** were recovered after aerating the irradiated reaction mixtures. A plausible mechanistic pathways for the formation of **1** from **9-11** are shown in Scheme 4.

Scheme 4



For **9** and **10**, the two-electron reduction of the *ortho*-quinonimine function occurs to give the hydroquinone intermediate **14**. The elimination of alcohol from **14** gives **1**, probably *via* the imino ketone intermediate **15**. The two-electron reduction of **11**, followed by elimination of water, gives **1** again *via* **15**. These results imply that compounds **9-11** may be used as candidates for RID. Work along this line is now in progress.

EXPERIMENTAL

Melting points were determined with a Buchi 510 capillary melting point apparatus and are uncorrected. The ^1H nmr spectra were recorded on a JEOL JNM-GX270 FT NMR spectrometer using TMS as the internal standard. Infrared spectra were recorded on a Shimadzu IR-4000 instrument. The purity of compounds was checked by tlc on silica-gel plates (Silica gel 60, F254, Merck). Elemental analyses were performed with a Yanagimoto CHN Corder MT-3. γ-Irradiations were carried out with a ^{137}Cs source at the National Institute of Genetics. The hplc analyses were performed on a Shimadzu LC-3A, using a 25 cm x 4 mm i.d. stainless steel column packed with a RP-18 chemically bonded silica gel (Lichrosorb, 10 μm, Merck).

Materials.

Mitomycin C was obtained from Kyowa Hakko Kogyo Co. Ltd. Compounds **9-13** were prepared by the methods reported in a previous paper [4]. Compound **6** and **7** were prepared from mitomycin C.

[1a*S*-(1aα,8β,8aα,8bα)]-6-Amino-8-{[(aminocarbonyl)oxy]methyl}-1,1a,2,8,8a,8b-hexahydro-8a-methoxy-5-methyl-1-(styryl)sulfonylazilidino[2′,3′:3,4]pyrrolo[1,2-*a*]indole-4,7-dione (**6**).

Styrylsulfonyl chloride (246 mg, 1.2 mmoles) was added to a suspension of mitomycin C (200 mg, 0.6 mmole) in pyridine (20 ml) and the mixture was stirred at room temperature for 1.5 hours. Chloroform (20 ml) was then added and the mixture was washed with aqueous sodium chloride solution, dried over sodium sulfate and concentrated. The residue was chromatographed on silica gel with chloroform-acetone (7:3) to give 230 mg (77%) of **6**, mp 180° dec: tlc (chloroform-acetone (1:1)): Rf 0.50; ir (potassium bromide): 3450, 3300, 1720, 1610, 1360 cm^{-1}; ^1H nmr (DMSO-d_6): δ 1.65 (3H, s), 2.70 (1H, bs), 2.79 (1H, bs), 3.15 (3H, s), 3.38 (1H, d), 3.50 (1H, dd), 4.00 (1H, d), 4.10 (1H, br), 4.55 (1H, dd), 6.50 (2H, bs), 7.00 (2H, br), 7.20 (1H, d), 7.40-7.50 (3H, m) 7.67-7.79 (2H, m), 7.90 (1H, d); ms: (m/z) 500 (M^+).

Anal. Calcd. for $C_{23}H_{24}N_4O_7S$: C, 55.20; H, 4.80; N, 11.20. Found: C, 55.02; H, 4.91; N, 11.05.

[1a*S*-(1aα,8β,8aα,8bα)]-1-(Allylamino)thiocarbonyl-6-amino-8-{[(aminocarbonyl)oxy]methyl}-1,1a,2,8,8a,8b-hexahydro-8a-methoxy-5-methylazilidino[2′,3′:3,4]pyrrolo[1,2-*a*]indole-4,7-dione (**7**).

Allyl isothiocyanate (357 mg, 3.6 moles) and 1-hydroxybenzotriazole (162 mg, 1.2 mmoles) were added to a suspension of mitomycin C (200 mg, 0.6 mmole) in pyridine (20 ml). The mixture was stirred at room temperature for 18 hours and concentrated. The residue was chromatographed on silica gel with chloroform-acetone (1:1) to give 150 mg (62%) of **7**, mp 195° dec; tlc (chloroform-acetone (1:1)) R_f 0.45; ir (potassium bromide) 3450, 3300, 1715, 1600, 1530 cm^{-1}; ^1H nmr (DMSO-d_6): δ 1.67 (3H, s), 2.75 (1H, bs), 2.82 (1H, bs), 3.12 (3H, s), 3.43 (1H, d), 3.54 (1H, dd), 3.88-4.29 (4H, m), 4.59 (1H, dd), 5.18-5.45 (2H, m), 5.61-5.85 (1H, m), 6.53 (2H, bs), 7.02 (2H, br), 8.45 (1H, bs); ms: (m/z) 401 (M^+).

Anal. Calcd. for $C_{19}H_{23}N_5O_5$: C, 56.86; H, 5.74; N, 17.46. Found: C, 56.69; H, 5.69; N, 17.29.

Competitive γ-Radiolyses of Mitomycin C (**1**) with 5-Fluoro-1-(phenylthioureido)uracil (**2**).

Sample solutions containing **1** in concentration of 200 μg/ml and varing amounts of **2** in aqueous 1% (v/v) methanol were prepared. Each of the sample solutions (2 ml) was placed in a 5 mm φ Pyrex glass tube and irradiated with γ-rays of 50 Gy at a dose rate of 3.15 Gy/minute at room temperature (*ca* 20°). After irradiation, the amounts of 5-FU, (**3**), produced were determined by hplc analysis.

The analysis conditions were as follows: Column, C_{18}-μ Bondapak; Mobile phase, 0.02 *M* KH_2PO_4-0.02 *M* K_2HPO_4-2% acetonitrile in a flow rate of 1 ml/minute; Detection, uv 270 nm. The rate constant for the reaction of **1** with e^-_{aq} was estimated by the use of Equation 6.

γ-Radiolysis of **6** and **7**.

Sample solutions (2 ml) containing **6** and **7** in the concentration of 200 μg/ml were irradiated with γ-rays of 50 Gy in a similar manner as above. After irradiation, the reaction mixtures were analysed for **1** by hplc (for the analysis conditions, see later).

γ-Radiolysis of **9-12**.

For each of compounds **9-12**, solutions were prepared by dissolving the compounds in deaerated aqueous 1% (v/v) methanol, in the concentration of 50 μg/ml. Each of the sample solutions (2 ml) was irradiated with γ-rays of 50 Gy at room temperature (*ca* 20°) in a similar manner as above. After irradiation, the amounts of **1** produced were determined by hplc analyses. The analysis conditions were as follows: Column, C_{18}-μ Bondapak; Mobile phase, 1/300 *M* phosphate buffer (*p*H 7.0)-methanol (8:3) in a flow rate of 1.0 ml/minute; detection, uv 254 nm. The G values for the formation of **1** were then calculated.

REFERENCES AND NOTES

[1] T. Kuroda, K. Hisamura, I. Matsukuma, H. Nishikawa and N. Nakamizo, *Bull. Chem. Soc. Japan*, **62**, 674 (1989).

[2] T. Kuroda, K. Hisamura, I. Matsukuma, H. Nishikawa, M. Morimoto, T. Ashizawa, N. Nakamizo and Y. Otsuzi, *J. Heterocyclic Chem.*, **29**, 1133 (1992).

[3] T. Kuroda, K. Hisamura, N. Nakamizo and Y. Otsuzi, *J. Heterocyclic Chem.*, **29**, 1143 (1992).

[4] T. Kuroda, K. Hisamura, I. Matsukuma, Y. Osawa, T. Sugaya, H. Nishikawa, M. Morimoto, T. Ashizawa, N. Nakamizo and Y. Otsuzi, *J. Heterocyclic Chem.*, Preceeding paper (submitted).

[5] T. Kuroda, K. Hisamura, N. Nakamizo and Y. Otsuzi, *Chem. Letters*, 707 (1989).

[6] M. Kondo and Y. Shinozaki, *Kiso Genshiryoku Koza*, Vol **7**, Hoshasen Kagaku, Corona Co. Ltd., Tokyo, 1980, p 230.

[7] R. T. Sanderson, Chemical Bonds and Bond Energy, Second Ed, Academic Press, New York, 1976, p 179.