[Chem. Pharm. Bull. 30(7)2534—2543(1982)]

The Stability of Carboquone in Aqueous Solution. II.[1] Kinetics and Mechanisms of Degradation of 2,5-Bis(1-aziridinyl)-3,6-dimethyl-1,4-benzoquinone and 2,5-Bis-(1-aziridinyl)-3,6-diisopropyl-1,4-benzoquinone in Aqueous Solution[2]

Akira Kusai,* Seiji Tanaka, and Seigo Ueda

Product Development Laboratories, Sankyo Co., Ltd., Hiromachi, Shinagawa-ku, Tokyo, 140, Japan

(Received November 20, 1981)

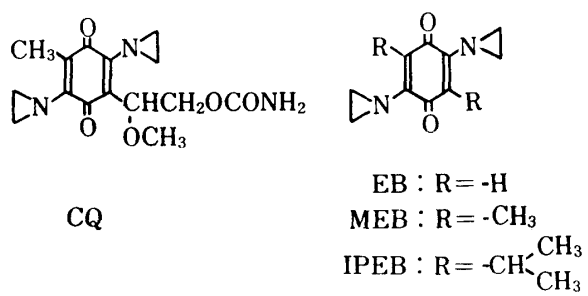
The kinetics and mechanisms of the degradation of 2,5-bis(1-aziridinyl)-3,6-dimethyl-1,4-benzoquinone (MEB) and 2,5-bis(1-aziridinyl)-3,6-diisopropyl-1,4-benzoquinone (IPEB) were investigated and compared with those of 2,5-bis(1-aziridinyl)-1,4-benzoquinone (EB) investigated previously. The degradation of MEB and IPEB follows pseudo first-order kinetics in the same way as that of EB. The pH-rate profiles showed slopes of −1 on the acidic side and +1 on the basic side, as did that of EB. Thus, the degradation of MEB and IPEB is subject to specific acid–base catalysis. The apparent activation energies for MEB degradation at pH 4 and pH 11 were 16 and 24 kcal/mol, and those for IPEB degradation were 17 and 23 kcal/mol, respectively.

In basic aqueous solution, MEB and IPEB are degraded to dihydroxybenzoquinones with monohydroxy-mono(1-aziridinyl)benzoquinones as intermediates in the same way as EB. On the other hand, in acidic aqueous solution, (2-hydroxyethylamino)benzoquinones are produced from MEB and IPEB, as in the case of EB, but they are further degraded to hydroxybenzoquinones. This was not practically observed in the case of EB. This phenomenon can be explained as follows: the alkyl groups at the 3 and 6 positions of benzoquinone increase the relative hydrolysis rate of 2-hydroxyethylamino groups derived from the hydrolytic cleavage of aziridine rings at the 2 and 5 positions of benzoquinone, making it comparable to the ring cleavage rate of aziridinyl groups.

Keywords——antitumor agent; (1-aziridinyl)benzoquinones; (2-hydroxyethylamino)-benzoquinones; hydroxybenzoquinones; kinetics and mechanisms; HPLC

Carboquone [CQ; 2,5-bis(1-aziridinyl)-3-(2-carbamoyloxy-1-methoxyethyl)-6-methyl-1,4-benzoquinone][3,4] is one of the antitumor agents with the (1-aziridinyl)benzoquinone structure (Chart 1) classified as alkylating agents.

In the previous report,[1] in order to investigate the degradation mechanism of CQ in aqueous solution, 2,5-bis(1-aziridinyl)-1,4-benzoquinone (EB, Chart 1) was chosen as a model compound of CQ, and the kinetics and mechanism of degradation of EB in aqueous solution were investigated by high pressure liquid chromatography (HPLC). It was found that EB follows pseudo first-order kinetics. From the pH-rate profile, it was concluded that the degradation of EB is subject to specific acid–base catalysis. In acidic media, EB is degraded to 2,5-bis(2-hydroxyethylamino)-1,4-benzoquinone with sequential hydrolytic cleavage of the two aziridine rings. In basic media, EB is degraded to 2,5-dihydroxy-1,4-benzoquinone with sequential substitution of the two aziridine rings by hydroxyl ion (radical).



CQ

EB : R = -H
MEB : R = -CH$_3$
IPEB : R = -CH(CH$_3$)$_2$

Chart 1

In the present report, the study was extended to 2,5-bis(1-aziridinyl)-3,6-dimethyl-1,4-benzoquinone (MEB or M-1, Chart 1) and 2,5-bis(1-aziridinyl)-3,6-diisopropyl-1,4-benzoquinone(IPEB or IP-1, Chart 1), and the kinetics and mechanisms

NII-Electronic Library Service

degradation in aqueous solutions were investigated and compared to the results obtained with EB.

Experimental

Materials——i) (1-Aziridinyl)benzoquinones: MEB was prepared according to Nakao *et al.*[3] (mp 213°C). *Anal.* Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.83. Found: C, 65.92; H, 6.44; N, 12.71. IPEB was also prepared according to Nakao *et al.*[3] (mp 170°C). *Anal.* Calcd for $C_{16}H_{22}N_2O_2$: C, 70.63; H, 8.08; N, 10.21. Found: C, 69.88; H, 8.06; N, 10.18.

ii) Bis(2-hydroxyethylamino)benzoquinones: 2,5-Bis(2-hydroxyethylamino)-1,4-benzoquinone (E-3) was prepared as described previously[1] (mp 266°C). *Anal.* Calcd for $C_{10}H_{14}N_2O_4$: C, 53.09; H, 6.24; N, 12.38. Found: C, 52.71; H, 6.16; N, 12.21.

2,5-Bis(2-hydroxyethylamino)-3,6-dimethyl-1,4-benzoquinone (M-3): To a solution of 1 g of 2,5-dimethyl-1,4-benzoquinone prepared according to Smith *et al.*[5] in 40 ml of 50% ether in ethanol, 0.6 ml of ethanolamine was added dropwise with stirring at room temperature. The resulting mixture was allowed to stand in a refrigerator for two days. The separated crystals were collected and recrystallized from ethanol to give 0.25 g of M-3 (mp 210°C). *Anal.* Calcd for $C_{12}H_{18}N_2O_4$: C, 56.68; H, 7.14; N, 11.02. Found: C, 56.48; H, 7.14; N, 10.88.

2,5-Bis(2-hydroxyethylamino)-3,6-diisopropyl-1,4-benzoquinone (IP-3): To 0.5 g of 2,5-diisopropyl-1,4-benzoquinone prepared according to Bogolybskii[6] in 20 ml of ethanol, 1 ml of ethanolamine was added. The mixture was refluxed at 80°C for 3 h. The reaction mixture was mixed with 500 ml of 0.01 M borate buffer (pH 9) at 0°C. The resulting precipitates were collected and recrystallized from 20% ethanol in H_2O to give 0.15 g of IP-3 (mp 166°C). *Anal.* Calcd for $C_{16}H_{26}N_2O_4$: C, 61.93; H, 8.38; N, 9.03. Found: C, 60.48; H, 8.20; N, 9.13.

iii) Dihydroxybenzoquinones: 2,5-Dihydroxy-3,6-dimethyl-1,4-benzoquinone (M-5): MEB (300 mg) was dissolved in 1 l of 0.3 N NaOH aqueous solution and stored at 100°C for 1 h, then the reaction mixture was acidified to pH 2 with HCl and extracted into ether. The extracts were concentrated under reduced pressure and the residue was recrystallized from ethanol to produce 80 mg of M-5 (mp 200°C). *Anal.* Calcd for $C_8H_8O_4$: C, 57.14; H, 4.80. Found: C, 57.10; H, 4.87; N, 0.00.

2,5-Dihydroxy-3,6-diisopropyl-1,4-benzoquinone (IP-5): IPEB (200 mg) was dissolved in 500 ml of 1 N NaOH aqueous solution and stored at 100°C for 1 h, then the reaction mixture was acidified to pH 2 with HCl and extracted into ether. The extract was concentrated under reduced pressure and the residue was recrystallized from ethanol to produce 40 mg of IP-5 (mp 157°C). *Anal.* Calcd for $C_{12}H_{16}O_4$: C, 64.29; H, 7.14. Found: C, 63.61; H, 7.10; N, 0.00.

Other chemicals used here were of the highest grade commercially available.

Buffer Solutions for Kinetic Study——Acetate buffer (pH 3—5), phosphate buffer (pH 6—8), borate buffer (pH 9) and carbonate buffer (pH 10—12.5) were used. The concentration of each buffer solution used was 0.01 M.

Procedure for Kinetic Study——i) Stock Solutions: Fifty milligrams of MEB, IPEB, M-3 or IP-3 was dissolved in *N*,*N*-dimethylacetamide and made up to 20 ml.

ii) Degradation Kinetics of MEB and IPEB: One milliliter aliquots of MEB or IPEB stock solution were mixed with 49 ml of buffer solutions equilibrated in a water bath thermostated at 20°, 30°, 40° or 50° ± 0.1°C. At regular intervals, 5 ml samples were taken into 20 ml volumetric flasks. To each flask, 1 ml of 0.5 M triethanolamine–acetate buffer solution, pH 7 (to stop the reaction), and 2 ml of methanol solution

Table I. HPLC Conditions

Method	Assayable compounds	Column	Mobile phase	Flow rate (ml/min)	Temp. (°C)	Internal standard
I	E-3	A	5% 10 mM $NaHCO_3$/MeOH	2.0	40	Methyl salicylate
II	M-1	A	10% 10 mM $NaHCO_3$/MeOH	2.0	40	Isopropyl salicylate
III	M-1, M-2 M-3	B	25% CH_3CN/50 mM $NaHCO_3$ (pH 8.5)	1.0	50	—
IV	M-4, M-5 M-6	B	10% MeOH/50 mM $NaHCO_3$ (pH 8.5)	1.0	50	—
V	IP-1	A	5% 10 mM $NaHCO_3$/MeOH	2.0	40	Isobutyl salicylate
VI	IP-1, IP-2 IP-3	B	50% CH_3CN/25 mM $NaHCO_3$ (pH 8.5)	1.0	50	—
VII	IP-4, IP-5 IP-6	A	40% 50 mM tri-ethanolamine/MeOH (pH 9.5)	0.8	40	—

NII-Electronic Library Service

of an internal standard were added, and the mixture was diluted to the mark with methanol. The solutions thus obtained were analyzed by means of HPLC (MEB, method II; IPEB, method V).

iii) Disappearance of MEB or IPEB and Appearance of Their Degradation Products: One milliliter aliquots of MEB or IPEB stock solution were mixed with 49 ml of buffer solutions equilibrated in a water bath thermostated at 50°C. Each of the 2 ml samples taken at regular intervals was mixed with 1 ml of 0.5 M sodium bicarbonate aqueous solution to stop the reaction. The solutions thus obtained were analyzed by means of HPLC (MEB, methods III and IV; IPEB, methods VI and VII).

iv) Degradation Kinetics of Bis(2-hydroxyethylamino)benzoquinones: The degradation kinetics of E-3 were followed by the same procedure as described previously for EB[1] (HPLC, method I). The degradation kinetics of M-3 and IP-3 were followed by the same procedure as described in section iii.

HPLC——Chromatography was performed on a Hitachi liquid chromatograph, model 635, equipped with a monitoring system operating at a wavelength of 325 nm. Column A (5 mm i.d. × 50 cm) of a porous styrene–divinylbenzene copolymer[7] and Column B (4 mm i.d. × 15 cm) of an octadecylsilane chemically bonded to totally porous silica gel[8] were used. Chromatographic conditions used here are listed in Table I.

Results and Discussion

Degradation Kinetics of MEB and IPEB

The degradation of MEB and that of IPEB follow pseudo first-order kinetics over a wide range of pH. Their pH–rate profiles are shown in Fig. 1. The slopes in the acidic region and in the basic region were −1 and +1, respectively. This indicates that the degradation of MEB and that of IPEB are subject to specific acid–base catalysis just as in the case of EB.[1] Apparent activation energies obtained from Arrhenius plots at pH 4 and pH 11 are listed in Table II. The values for MEB and IPEB show a relationship similar to those for EB.[1]

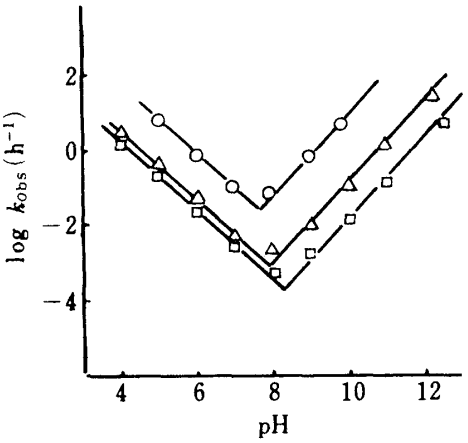


Fig. 1. log k_{obs}–pH Profiles for the Degradation of EB (○), MEB (△) and IPEB (□) at 50°C

TABLE II. Apparent Activation Energies obtained from Arrhenius Plots

Compound	Activation energy (kcal/mol)	
	pH 4	pH 11
MEB	16	24
IPEB	17	23
EB	14	19

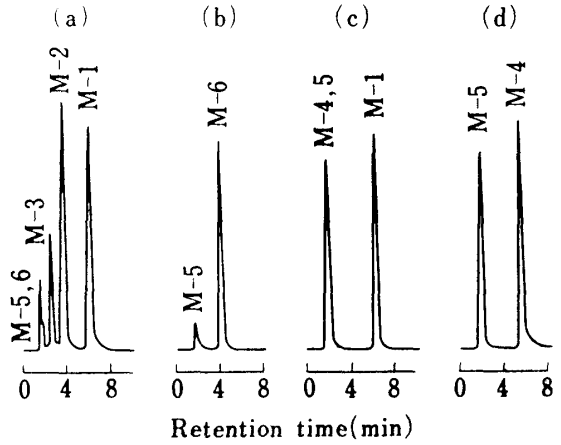


Fig. 2. Chromatograms obtained from Aqueous Solutions of MEB by HPLC (method III or IV)

(a) from 0.01 M acetate buffer, pH 3, after 2 min at 50°C. The remaining MEB is 35.5% (method III).
(b) from 0.01 M acetate buffer, pH 3, after 30 min at50°C. The remaining MEB is 0%. (method IV).
(c) from 0.01 M carbonate buffer, PH 12, after 5 min at 50°C. The remaining MEB is 50% (method III).
(d) from 0.01 M carbonate buffer, pH 12, after 60 min at 50°C. The remaining MEB is 0% (method IV).

In the case of HPLC method IV, M-3 appears at around 30 min as a broad peak, and M-1 and M-2 do not show any distinct peak due to the long retention times in the column.

M-1: 2,5-bis(1-aziridinyl)-3,6-dimethyl-1,4-benzoquinone.
M-2: 2-(2-hydroxyethylamino)-5-(1-aziridinyl)-3,6-dimethyl-1,4-benzoquinone.
M-3: 2,5-bis(2-hydroxyethylamino)-3,6-dimethyl-1,4-benzoquinone.
M-4: 2-hydroxy-5-(1-aziridinyl)-3,6-dimethyl-1,4-benzoquinone.
M-5: 2,5-dihydroxy-3,6-dimethyl-1,4-benzoquinone.
M-6: 2-hydroxy-5-(2-hydroxyethylamino)-3,6-dimethyl-1,4-benzoquinone.

NII-Electronic Library Service

Degradation Products of MEB in Aqueous Solution

HPLC patterns for MEB stored in buffers at pH 3 and pH 12, and the time course of each peak are shown in Figs. 2 and 3, respectively. Four peaks are observed as degradation products under acidic conditions and 2 peaks under basic conditions. M-3 and M-5 were identified as 2,5-bis(2-hydroxyethylamino)-3,6-dimethyl-1,4-benzoquinone and 2,5-dihydroxy-3,6-dimethyl-1,4-benzoquinone; their HPLC retention times and UV spectra after HPLC separation coincided well with those of authentic samples. M-6 appeared on acidifying the solution containing M-4.[9] M-5 and M-6 were observed as degradation products of M-3 in aqueous solution. These results and the result for EB[1] indicate that M-2, M-4 and M-6 are 2-(2-hydroxyethylamino)-5-(1-aziridinyl)-3,6-dimethyl-1,4-benzoquinone, 2-hydroxy-5-(1-aziridinyl)-3,6-dimethyl-1,4-benzoquinone and 2-hydroxy-5-(2-hydroxyethylamino)-3,6-dimethyl-1,4-benzoquinone, respectively.

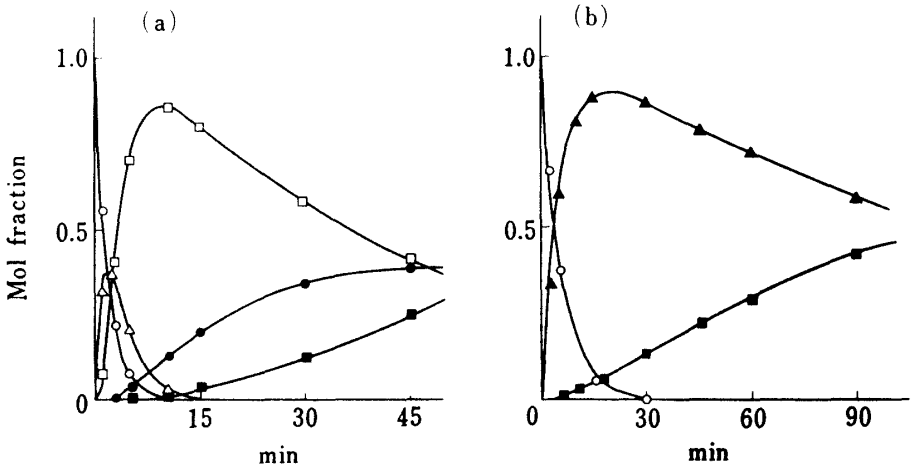


Fig. 3. Time Courses of M-1 (○), M-2 (△) M-3 (□), M-4 (▲), M-5 (■) and M-6 (●) during MEB Degradation at 50°C

(a) in 0.01 M acetate buffer, pH 3.
(b) in 0.01 M carbonate buffer, pH 12.
The lines are calculated values based on Chart 4.

Degradation Mechanisms of MEB in Aqueous Solution

The degradation products, their time courses and the result for EB[1] suggest that the degradation mechanism of MEB in basic aqueous solution is as shown in Chart 2. When MEB is degraded according to Chart 2, the mol fractions of M-1, M-4 and M-5 at time t can be calculated from equations 1, 2 and 3, respectively.[10]

$$\text{M-1} \xrightarrow{k_3} \text{M-4} \xrightarrow{k_4} \text{M-5}$$

Chart 2

$$X(\text{M-1}_t) = \exp(-k_3 t) \qquad \text{Eq. 1}$$

$$X(\text{M-4}_t) = \frac{k_3}{k_4 - k_3}\{\exp(-k_3 t) - \exp(-k_4 t)\} \qquad \text{Eq. 2}$$

$$X(\text{M-5}_t) = 1 + \frac{1}{k_3 - k_4}\{k_4 \exp(-k_3 t) - k_3 \exp(-k_4 t)\} \qquad \text{Eq. 3}$$

Experimentally, the mol fractions of M-1 and M-5 at time t can be obtained from HPLC data. The mol fraction of M-4 can be calculated from equation 4.

$$X(\text{M-4}_t) = 1 - \{X(\text{M-1}_t) + X(\text{M-5}_t)\} \qquad \text{Eq. 4}$$

NII-Electronic Library Service

$$\text{M-1} \xrightarrow{k_1} \text{M-2} \xrightarrow{k_2} \text{M-3} \xrightarrow{k_5} \text{M-6} \xrightarrow{k_6} \text{M-5}$$

Chart 3

As shown in Fig. 3(b), the calculated values agreed well with the observed values, assuming $k_3=10.8\ h^{-1}$ and $k_4=0.381\ h^{-1}$.

On the other hand, the degradation products and their time courses suggest that the degradation mechanism of MEB in acidic solution is as shown in Chart 3. When MEB is degraded according to Chart 3, the mol fractions of M-1, M-2, M-3, M-6 and M-5 at time t can be calculated from equations 5, 6 (or 6'), 7 (or 7'), 8 (or 8') and 9, respectively.

$$X(\text{M-1}_t) = \exp(-k_1 t) \qquad \text{Eq. 5}$$

$$X(\text{M-2}_t) = \frac{k_1}{k_2-k_1}\{\exp(-k_1 t)-\exp(-k_2 t)\} \qquad \text{Eq. 6}$$

$$X(\text{M-3}_t) = \frac{k_1 k_2}{k_2-k_1}\left\{\frac{\exp(-k_1 t)-\exp(-k_5 t)}{k_5-k_1} - \frac{\exp(-k_2 t)-\exp(-k_5 t)}{k_5-k_2}\right\} \qquad \text{Ep. 7}$$

$$X(\text{M-6}_t) = \frac{k_1 k_2 k_5}{k_2-k_1}\left[\frac{1}{k_5-k_1}\left\{\frac{\exp(-k_1 t)-\exp(-k_6 t)}{k_6-k_1} - \frac{\exp(-k_5 t)-\exp(-k_6 t)}{k_6-k_5}\right\} - \frac{1}{k_5-k_2}\left\{\frac{\exp(-k_2 t)-\exp(-k_6 t)}{k_6-k_2} - \frac{\exp(-k_5 t)-\exp(-k_6 t)}{k_6-k_5}\right\}\right] \qquad \text{Eq. 8}$$

$$X(\text{M-5}_t) = 1-\{X(\text{M-1}_t)+X(\text{M-2}_t)+X(\text{M-3}_t)+X(\text{M-6}_t)\} \qquad \text{Eq. 9}$$

When k_1 is equal to k_2, and k_5 is equal to k_6,

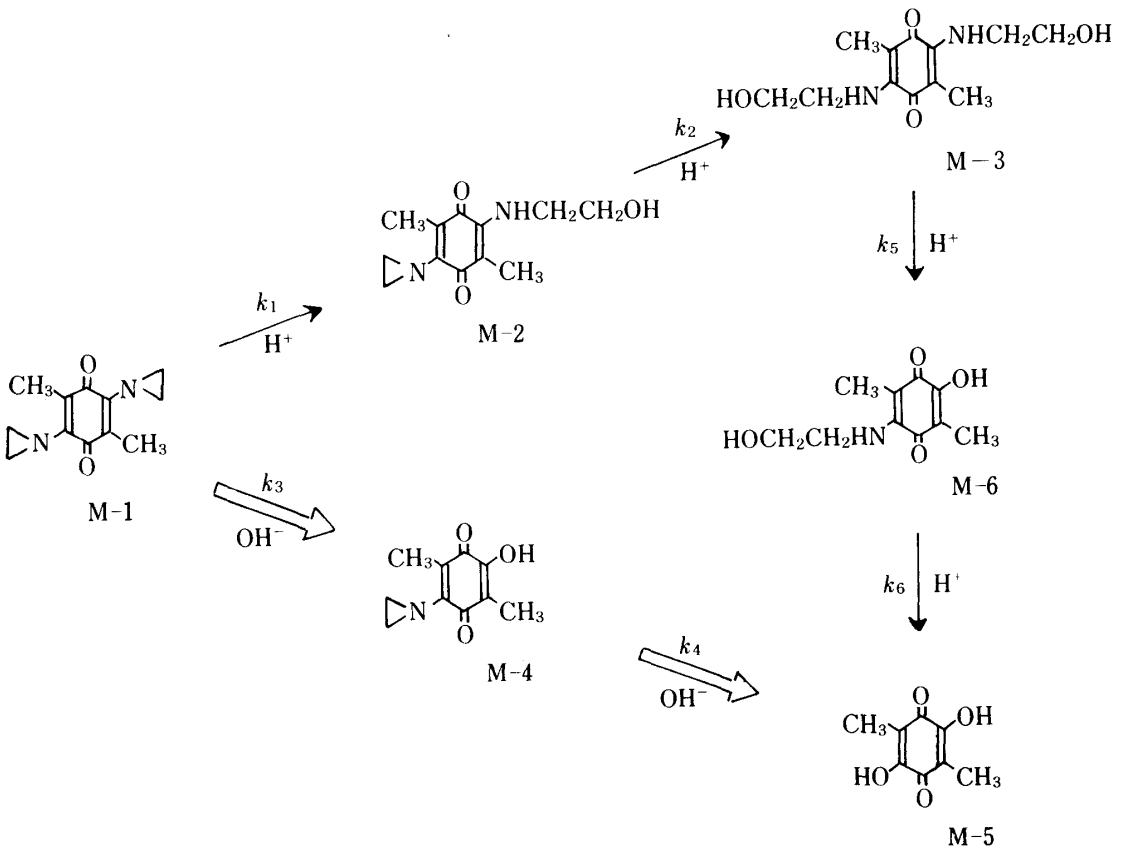


Chart 4

⇨: basic aqueous solution,
→: acidic aqueous solution.

NII-Electronic Library Service

$$X(\text{M-2}_t) = k_1 t \exp(-k_1 t) \qquad \text{Eq. 6'}$$

$$X(\text{M-3}_t) = \frac{k_1^2}{k_5 - k_1}\left\{t \exp(-k_1 t) - \frac{\exp(-k_1 t) - \exp(-k_5 t)}{k_5 - k_1}\right\} \qquad \text{Ep. 7'}$$

$$X(\text{M-6}_t) = \frac{k_1^2 k_5}{k_5 - k_1}\left[t\{\exp(-k_1 t) + \exp(-k_5 t)\} - \frac{2\{\exp(-k_1 t) - \exp(-k_5 t)\}}{k_5 - k_1}\right] \qquad \text{Eq. 8'}$$

Experimentally, the mol fractions of M-1, M-3 and M-5 at time t can be obtained from HPLC data, using authentic compounds. The mol fraction of M-2 at time t can be calculated from the data, using the specific peak height obtained by HPLC of a sample solution at an initial stage of reaction when only M-2 and M-3 are observed as degradation products. Further, the mol fraction of M-6 at time t can be calculated from equation 10,

$$X(\text{M-6}_t) = 1 - \{X(\text{M-1}_t) + X(\text{M-2}_t) + X(\text{M-3}_t) + X(\text{M-5}_t)\} \qquad \text{Eq. 10}$$

As shown in Fig. 3(a), the calculated values agree well with the observed values, assuming $k_1 = k_2 = 31.05\ \text{h}^{-1}$ and $k_5 = k_6 = 1.35\ \text{h}^{-1}$. It may be noted that for short times, *i.e.* up to the half-life of MEB, the simpler Chart, M-1→M-2→M-3, would adequately describe the system.

Therefore, the degradation mechanisms of MEB in aqueous solution are concluded to be as shown in Chart 4: In basic aqueous solution (⇨), MEB is degraded to the dihydroxy compound (M-5) in just the same way as EB.[1] In acidic aqueous solution (→), MEB is degraded to the bis(2-hydroxyethylamino) compound (M-3) in the same way as EB,[1] but this ring cleavage reaction is further followed by the hydrolysis of 2-hydroxyethylamino groups, which was not practically observed in the case of EB.[1] These phenomena may be due to an increase

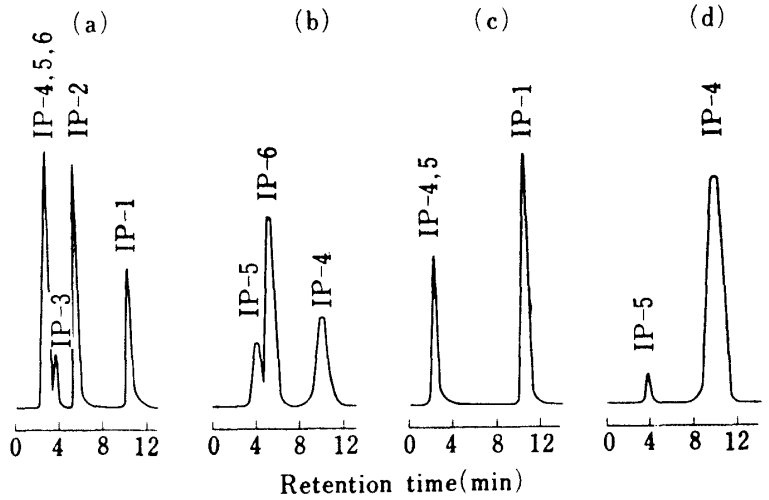


Fig. 4. Chromatograms obtained from Aqueous Solutions of IPEB by HPLC (Method VI or VII)

(a) from 0.01 M acetate buffer, pH 4, after 60 min at 50°C. The remaining IPEB is 18% (method VI).
(b) from 0.01 M acetate buffer, pH 4, after 60 min at 50°C. The remaining IPEB is 18% (method VII).
(c) from 0.01 M carbonate buffer, pH 12.5, after 5 min at 50°C. The remaining IPEB is 63% (method VI).
(d) from 0.01 M carbonate buffer, pH 12.5, after 120 min at 50°C. The remain ing IPEB is 0% (method VII).

In the case of HPLC method VII, IP-3 appears at around 45 min as a broad peak, and IP-1 and IP-2 do not show any distinct peak due to the long retention times in the columu.

IP-1: 2,5-bis(1-aziridinyl)-3,6-diisopropyl-1,4-benzoquinone.
IP-2: 2-(2-hydroxyethylamino)-5-(1-aziridinyl)-3,6-diisopropyl-1,4-benzoquinone.
IP-3: 2,5-bis(2-hydroxyethylamino)-3,6-diisopropyl-1,4-benzoquinone.
IP-4: 2-hydroxy-5-(1-aziridinyl)-3,6-diisopropyl-1,4-benzoquinone.
IP-5: 2,5-dihydroxy-3,6-diisopropyl-1,4-benzoquinone.
IP-6: 2-hydroxy-5-(2-hydroxyethylamino)-3,6-diisopropyl-1,4-benzoquinone.

NII-Electronic Library Service

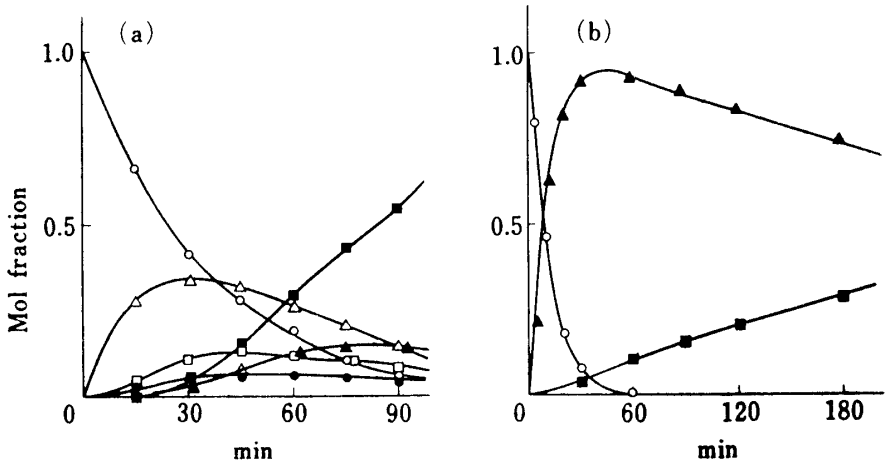


Fig. 5. Time Courses of IP-1 (○), IP-2 (△), IP-3 (□), IP-4 (▲), IP-5 (■) and IP-6 (●) during IPEB Degradation at 50°C

(a) in 0.01 M acetate buffer, pH 4.
(b) in 0.01 M carbonate buffer, pH 12.5.
The lines are calculated values based on Chart 7.

in the lability of 2-hydroxyethylamino groups of M-3 relative to the lability of aziridine rings of MEB and M-2.

Degradation Products of IPEB in Aqueous Solution

HPLC patterns of IPEB stored in buffers at pH 4 and pH 12.5, and the time course of each peak are shown in Figs. 4 and 5, respectively. Five peaks are observed as degradation products under acidic conditions and 2 peaks under basic conditions. IP-3 and IP-5 were identified as 2,5-bis(2-hydroxyethylamino)-3,6-diisopropyl-1,4-benzoquinone and 2,5-dihydroxy-3,6-diisopropyl-1,4-benzoquinone from their HPLC retention times and UV spectra after HPLC separation in comparison with those of authentic samples. IP-5 and IP-6 were observed as degradation products of IP-3 in aqueous solution. These results and the results for EB[1] and MEB indicate that IP-2, IP-4 and IP-6 are 2-(2-hydroxyethylamino)-5-(1-aziridinyl)-3,6-diisopropyl-1,4-benzoquinone, 2-hydroxy-5-(1-aziridinyl)-3,6-diisopropyl-1,4-benzoquinone and 2-hydroxy-5-(2-hydroxyethylamino)-3,6-diisopropyl-1,4-benzoquinone, respectively.

Degradation Mechanisms of IPEB in Aqueous Solution

The degradation products, their time courses and the results for EB[1] and MEB suggest that the degradation mechanism of IPEB in basic aqueous solution is as shown in Chart 5.

$$\text{IP-1} \xrightarrow{k_3} \text{IP-4} \xrightarrow{k_4} \text{IP-5}$$

Chart 5

When IPEB is degraded according to Chart 5, the mol fractions of IP-1, IP-4 and IP-5 at time t can be calculated using equations 1, 2 and 3, as in the case of MEB.

Experimentally, the mol fractions of IP-1 and IP-5 at time t can be obtained from HPLC data. The mol fraction of IP-4 at time t can be calculated from equation 4 just as in the case of MEB.

As shown in Fig. 5(b), the calculated values agree well with the observed values, assuming $k_3=5.52\ \text{h}^{-1}$ and $k_4=0.115\ \text{h}^{-1}$.

$$\text{IP-1} \xrightarrow{k_1} \text{IP-2} \begin{array}{l} \xrightarrow{k_2} \text{IP-3} \\ \xrightarrow{k_7} \text{IP-4} \end{array} \begin{array}{l} \xrightarrow{k_5} \\ \xrightarrow{k_8} \end{array} \text{IP-6} \xrightarrow{k_6} \text{IP-5}$$

Chart 6

NII-Electronic Library Service

On the other hand, the degradation products and their time courses suggest that the degradation mechanism of IPEB in acidic aqueous solution is as shown in Chart 6. When IPEB is degraded according to Chart 6, the mol fractions of IP-1, IP-2, IP-3, IP-4, IP-6 and IP-5 at time t can be calculated from equations 11, 12, 13, 14, 15 and 16, respectively.

$$X(\text{IP-1}_t) = \exp(-k_1 t) \qquad \text{Eq. 11}$$

$$X(\text{IP-2}_t) = \frac{k_1}{(k_2+k_7)-k_1}\{\exp(-k_1 t)-\exp(-(k_2+k_7)t)\} \qquad \text{Eq. 12}$$

$$X(\text{IP-3}_t) = \frac{k_1 k_2}{(k_2+k_7)-k_1}\left\{\frac{\exp(-k_1 t)-\exp(-k_5 t)}{k_5-k_1} - \frac{\exp(-(k_2+k_7)t)-\exp(-k_5 t)}{k_5-(k_2+k_7)}\right\} \qquad \text{Eq. 13}$$

$$X(\text{IP-4}_t) = \frac{k_1 k_7}{(k_2+k_7)-k_1}\left\{\frac{\exp(-k_1 t)-\exp(-k_8 t)}{k_8-k_1} - \frac{\exp(-(k_2+k_7)t)-\exp(-k_8 t)}{k_8-(k_2+k_7)}\right\} \qquad \text{Eq. 14}$$

$$X(\text{IP-6}_t) = \frac{k_1 k_2 k_5}{(k_2+k_7)-k_1}\left[\frac{1}{k_5-k_1}\left\{\frac{\exp(-k_1 t)-\exp(-k_6 t)}{k_6-k_1} - \frac{\exp(-k_5 t)-\exp(-k_6 t)}{k_6-k_5}\right\} - \frac{1}{k_5-(k_2+k_7)}\left\{\frac{\exp(-(k_2+k_7)t)-\exp(-k_6 t)}{k_6-(k_2+k_7)} - \frac{\exp(-k_5 t)-\exp(-k_6 t)}{k_6-k_5}\right\}\right] + \frac{k_1 k_7 k_8}{(k_2+k_7)-k_1}\left[\frac{1}{k_8-k_1}\left\{\frac{\exp(-k_1 t)-\exp(-k_6 t)}{k_6-k_1} - \frac{\exp(-k_8 t)-\exp(-k_6 t)}{k_6-k_8}\right\} - \frac{1}{k_8-(k_2+k_7)}\left\{\frac{\exp(-(k_2+k_7)t)-\exp(-k_6 t)}{k_6-(k_2+k_7)} - \frac{\exp(-k_8 t)-\exp(-k_6 t)}{k_6-k_8}\right\}\right] \qquad \text{Eq. 15}$$

$$X(\text{IP-5}_t) = 1 - \{X(\text{IP-1}_t) + X(\text{IP-2}_t) + X(\text{IP-3}_t) + X(\text{IP-4}_t) + X(\text{IP-6}_t)\} \qquad \text{Eq. 16}$$

Experimentally, the mol fractions of IP-1, IP-3 and IP-5 at time t can be obtained from HPLC data, using authentic compounds. The mol fraction of IP-4 at time t can be calculated from the data, using the specific peak height obtained from HPLC of a sample solution at pH 12.5. The mol fraction of IP-2 at time t can be calculated from the data, using the specific peak height obtained by HPLC of a sample solution at an initial stage of reaction ($\sim$15 min) when only IP-2, IP-3 and IP-4 are observed as degradation products. Further, the mol fraction of IP-6 at time t can be calculated from equation 17,

$$X(\text{IP-6}_t) = 1 - \{X(\text{IP-1}_t) + X(\text{IP-2}_t) + X(\text{IP-3}_t) + X(\text{IP-4}_t) + X(\text{IP-5}_t)\} \qquad \text{Eq. 17}$$

As shown in Fig. 5(a), the calculated values agree well with the observed values, assuming $k_1=k_2=k_8=1.75$ h^{-1}, $k_5=k_6=4.2$ h^{-1} and $k_7=0.5$ h^{-1}, respectively.

Therefore, the degradation mechanisms of IPEB in aqueous solution are concluded to be as shown in Chart 7. In basic aqueous solution ($\Rightarrow$), IPEB is degraded to the dihydroxy compound (IP-5) in just the same way as EB[1] and MEB. In acidic aqueous solution ($\rightarrow$), the aziridine rings of IPEB are cleaved to produce a mono(2-hydroxyethylamino)-mono-(1-aziridinyl) compound (IP-2) and the bis(2-hydroxyethylamino) compound (IP-3) as in the case of EB,[1] but IP-3 is further hydrolyzed to produce IP-6 and IP-5 consecutively, as was observed in the case of MEB but not in the case of EB.[1] Also, a monohydroxy-mono(1-aziridinyl) compound (IP-4) is produced from IP-2, which was not practically observed in the cases of EB[1] and MEB. These phenomena may be due to the increase in the lability of 2-hydroxy-

NII-Electronic Library Service

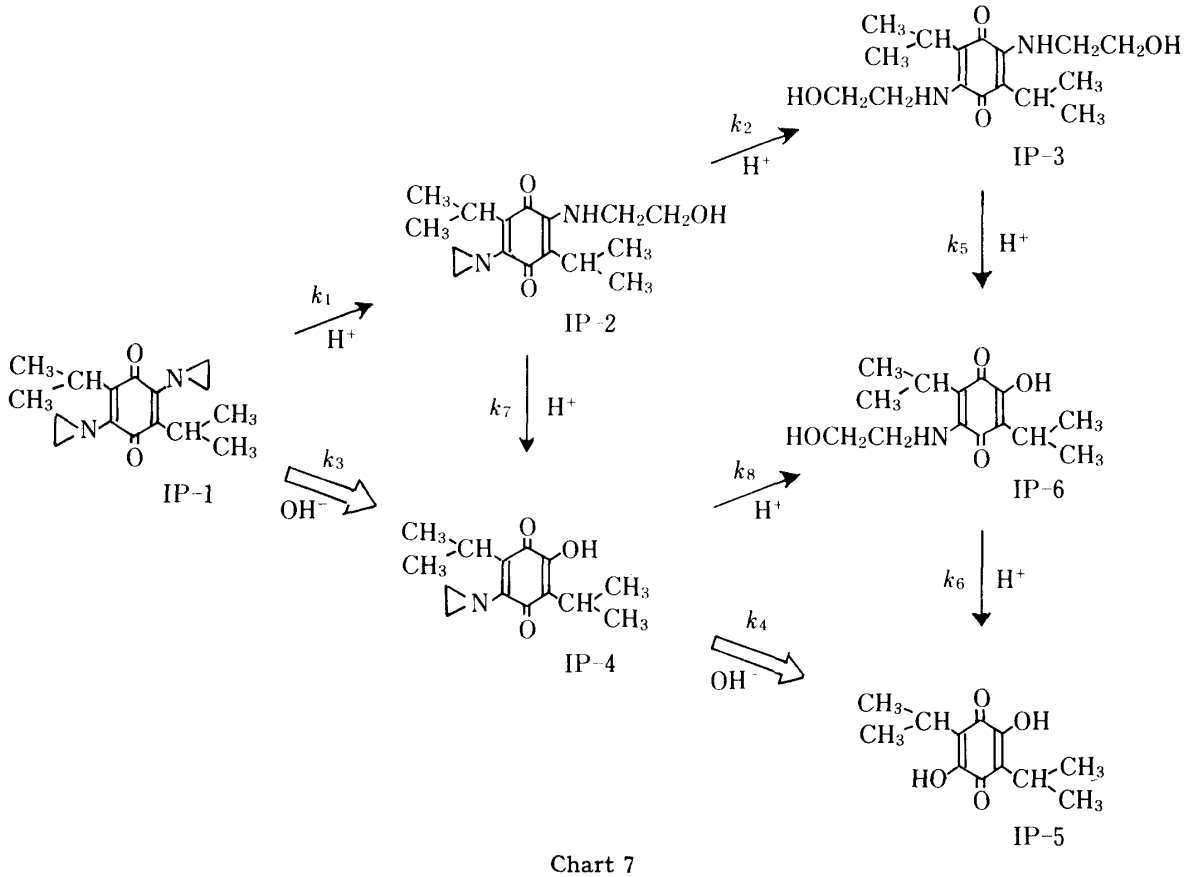


Chart 7

⇨: basic aqueous solution,
→: acidic aqueous solution.

ethylamino groups of IP-2 and IP-3 relative to the lability of the aziridine rings of IPEB and IP-2.

Degradation Kinetics of Bis(2-hydroxyethylamino)benzoquinone

The degradation mechanisms of MEB and IPEB in acidic aqueous solution are more complicated than that of EB. The hydrolysis rate of the 2-hydroxyethylamino group derived from the hydrolytic cleavage of the aziridine ring seems to be increased to a level comparable to the ring cleavage rate of the aziridinyl group by the presence of alkyl groups substituted at the 3 and 6 positions of EB. Therefore, the degradation kinetics of bis(2-hydroxyethylamino)-benzoquinones were investigated in order to confirm this assumption. pH–rate profiles of the degradation of E-3, M-3 and IP-3 are shown in Fig. 6. These results support the above assumption.

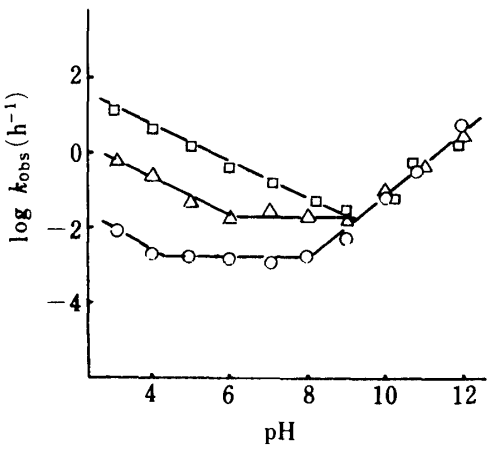


Fig. 6. log k_{obs}–pH Profiles for the Degradation of E-3 (○), M-3 (△), and IP-3 (□) at 50°C

At pH 4, for example, the ratio of apparent rate constant of (1-aziridinyl)benzoquinones to that of (2-hydroxyethylamino)benzoquinone is about 3×10^4 when R=H, about 10 when R=CH_3, and 0.4 when R=$CH(CH_3)_2$. Therefore, E-3 can be regarded as essentially inert in comparison with EB. When R=CH_3, the hydrolysis of 2-hydroxyethylamino groups becomes mea-

NII-Electronic Library Service

surable, but is still much slower than the hydrolysis of aziridine rings. The reaction of M-2 to M-4 was not experimentally observed, in other words, M-2 is hydrolyzed to M-3 before M-4 is produced from M-2. When $R=CH(CH_3)_2$, the hydrolysis of 2-hydroxyethylamino groups becomes faster than that of aziridine rings. The formation of IP-4 from IP-2 was observed.

Acknowledgement The authors are grateful to Dr. S. Akagi, director of these laboratories, and Dr. R. Okada for their kind encouragement and for permission to publish this article. Thanks are also due to Dr. W.I. Higuchi, College of Pharmacy, the University of Michigan, for many helpful suggestions during the preparation of this manuscript.

References and Notes

1) Part I: A. Kusai, S. Tanaka, and S. Ueda, *Chem. Pharm. Bull.*, **29**, 3671 (1981).
2) A part of this work was presented at the 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1980.
3) H. Nakao, M. Arakawa, T. Nakamura, and M. Fukushima, *Chem. Pharm. Bull.*, **20**, 1968 (1972).
4) H. Shindo, K. Nambu, E. Nakajima, and K. Kawai, *Yakugaku Zasshi*, **94**, 1393 (1974).
5) D. Smith and G. Gilbert, *J. Chem. Soc.*, **1964**, 873.
6) V.A. Bogolybskii, *Zh. Obshch. Khim.*, **32**, 869 (1962) [*Chem. Abstr.*, **58**, 2391*d* (1963)].
7) Hitachi gel 3010 (Hitachi Ltd., Tokyo, Japan).
8) LiChrosorb RP-18 (E. Merck, Darmstadt, Germany).
9) Aqueous solution containing M-4 can be obtained from a basic aqueous solution of MEB at an early stage of the reaction by chloroform extraction.
10) A.A. Frost and R.G. Pearson, "Kinetics and Mechanisms," John Wiley and Sons, Inc., New York, 1953, p. 153.

NII-Electronic Library Service