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The Stability of Carboquone in Alcohols. I. Kinetics and Mechanisms of Degradation of 2,5-Bis(1-aziridinyl)-1,4-benzoquinone in Alcohols

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The kinetics and mechanisms of the degradation of 2,5-bis(1-aziridinyl)-1,4-benzoquinone (I) in alcohols were investigated and compared with those in aqueous solution obtained previously. The degradation of I in alcohols follows pseudo first-order kinetics as did that in aqueous solution. Degradation rates in a series of alcohols show a good correlation with Kosower's *Z*-values (empirical measures of solvent polarity).

Degradation mechanisms of I in alcohols were investigated in methanol, where the degradation rate of I is relatively high, being suitable for detailed study. Five compounds were observed as degradation products of I in methanol, and the degradation process of I appeared to be rather complicated. However, the degradation behavior of I and some of these five degradation products in proton-rich methanol and methylate-rich methanol allowed us to deduce the chemical structures of all the degradation products: 5-(1-aziridinyl)-2-(2-methoxyethyl)amino-1,4-benzoquinone, 2,5-di(2-methoxyethyl)amino-1,4-benzoquinone, 5-(1-aziridinyl)-2-methoxy-1,4-benzoquinone, 2,5-dimethoxy-1,4-benzoquinone and 5-methoxy-2-(2-methoxyethyl)amino-1,4-benzoquinone. Their behavior also made it clear that I is degraded in methanol through a combination of two mechanisms: methanolysis of the aziridine ring, which is cleaved to a methoxyethylamino group, and substitution of the aziridine ring by a methoxy group. Therefore, the degradation mechanisms of I in methanol are concluded to be essentially the same as those in aqueous solution.

Keywords—antitumor agent; aziridinylbenzoquinone; methoxyethylaminobenzoquinone; methoxybenzoquinone; kinetics; mechanism; methanolysis; ring cleavage; Michael reaction; *Z*-value; HPLC

Carboquone [CQ; 2,5-bis(1-aziridinyl)-3-(2-carbamoyloxy-1-methoxyethyl)-6-methyl-1,4-benzoquinone]^{1,2)} is one of the antitumor agents with the (1-aziridinyl)benzoquinone structure (Chart 1), which are classified as alkylating agents. CQ is fairly unstable in protic solvents such as water and alcohols, and this may be mainly due to the lability of the aziridine moiety in the structure.

In three previous reports,³⁻⁵⁾ the kinetics and mechanisms of the degradation of CQ and related compounds, 2,5-bis(1-aziridinyl)-3,6-dialkyl-1,4-benzoquinones, in aqueous solution were studied. These compounds were found to undergo changes of the two aziridine rings: hydrolytic cleavage of them in acidic solutions, substitution of them by hydroxyl ion (radical)

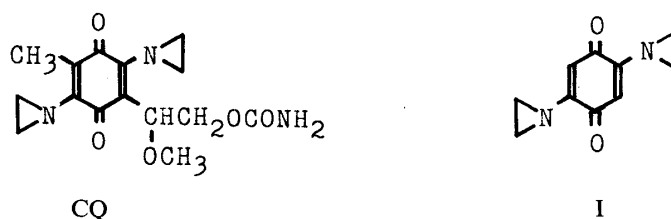


Chart 1

in basic solutions, and a combination of these two mechanisms in solutions of neutral pH.

Our studies have now been extended to the degradation behavior of CQ and related compounds in alcohols. In the present report, 2,5-bis(1-aziridiny)-1,4-benzoquinone (I, Chart 1) was chosen because of its simple structure and high reactivity, and its degradation kinetics and mechanisms in alcohols were investigated.

Experimental

Materials—I was prepared according to Gauss *et al.*⁶⁾ (mp 203 °C). *Anal.* Calcd for C₁₀H₁₀N₂O₂: C, 63.14; H, 5.30; N, 14.73. Found: C, 62.83; H, 5.29; N, 14.70.

2,5-Dimethoxy-1,4-benzoquinone was prepared according to Benington *et al.*⁷⁾ (mp 230 °C). *Anal.* Calcd for C₈H₈O₄: C, 57.14; H, 4.76. Found: C, 56.87; H, 4.81.

Other chemicals used were of the highest grade commercially available.

Procedure for Kinetic Study—Stock Solution: I (100 mg) was dissolved in *N,N*-dimethylacetamide (DMA)⁸⁾ and made up to 20 ml.

Degradation Kinetics of I: The stock solution of I (1 ml) was diluted to 100 ml with a given alcohol. Solutions thus obtained were placed in glass ampoules (2 ml). After being sealed, the ampoules were stored in a water bath thermostated at 60 °C, and taken out at regular intervals for high performance liquid chromatography (HPLC) assay.

Degradation Study of I in Proton-Rich Methanol and Methylate-Rich Methanol: Sulfuric acid and sodium methylate (28% in methanol) were used to make methanol proton-rich and methylate-rich, respectively. The stock solution of I (1 ml) was diluted to 100 ml with these treated methanols preincubated in a water bath thermostated at 60 °C. Two milliliter samples of the reaction mixture taken at regular intervals were each mixed with 2 ml of 10% 0.1 M triethanol-acetate (pH 7) in methanol to stop the reaction. The solutions thus obtained were loaded onto an HPLC column.

Isolation of Some Degradation Products of I in Methanol—III: Chloroform solution of I (250 mg/50 ml) was mixed with 200 ml of proton-rich methanol equilibrated at 60 °C (apparent proton concentration was adjusted to 10⁻³ M). After 2 min storage, the reaction mixture was poured into a separatory funnel containing 200 ml of chloroform and 250 ml of distilled water, and shaken vigorously. After standing for 10 min, the chloroform layer was collected and evaporated to dryness. The residue was recrystallized from ethanol to yield 160 mg of III (mp 128 °C).

II: Chloroform solution of I (25 mg/5 ml) was mixed with 20 ml of proton-rich methanol at room temperature (apparent proton concentration was adjusted to 5 × 10⁻⁵ M). After 1 min storage, the reaction mixture was mixed with 20 ml of chloroform and 25 ml of distilled water. The chloroform layer was collected and loaded onto silica gel thin-layer chromatography (TLC) plates,⁹⁾ which were developed in chloroform-ethanol (10:1). After development, the areas corresponding to *R_f* 0.7 were collected and extracted with 20% ethanol in chloroform. The extract was evaporated to dryness and the residue was dissolved in DMA. The DMA solution of II thus obtained was used to study the degradation behavior in methanol.

IV: Chloroform solution of I (25 mg/5 ml) was mixed with 20 ml of methylate-rich methanol equilibrated in a water bath thermostated at 60 °C (apparent methylate concentration was adjusted to 10⁻³ M). After 2 h storage, the reaction mixture was mixed with 20 ml of chloroform and 25 ml of distilled water. The chloroform layer was collected and concentrated under reduced pressure. The solution thus obtained was loaded onto a Lobar prepacked column.¹⁰⁾ The mobile phase, the flow rate and the temperature were 15% acetonitrile in water, 1 ml/min and room temperature, respectively. The fractions corresponding to IV (eluates from 35 to 45 min) were collected and extracted into chloroform. The chloroform layer thus obtained was evaporated to dryness and the residue was dissolved in DMA. The DMA solution of IV thus obtained was used to study the degradation behavior in methanol.

HPLC—Chromatography was performed on a Hitachi liquid chromatograph, model 655, with a variable-wavelength monitoring system (330 nm was used unless otherwise stated). A column (6 mm i.d. × 10 cm) of an octadecylsilane chemically bonded to totally porous silica gel¹¹⁾ was used. The mobile phase was 15% acetonitrile in water. The flow rate and the temperature were 1.7 ml/min and 40 °C, respectively.

Results and Discussion

Degradation Kinetics of I

The degradation of I follows pseudo first-order kinetics in a given alcohol. The degradation rates of I in a series of alcohols give a fairly good straight line when plotted against Kosower's *Z*-value,¹²⁾ which is an empirical measure of solvent polarity based on the position of the charge transfer band of 1-ethyl-4-carbomethoxypyridinium iodide (Fig. 1). Rate constants of I in water and alcohols containing water also lay on the line, which suggest

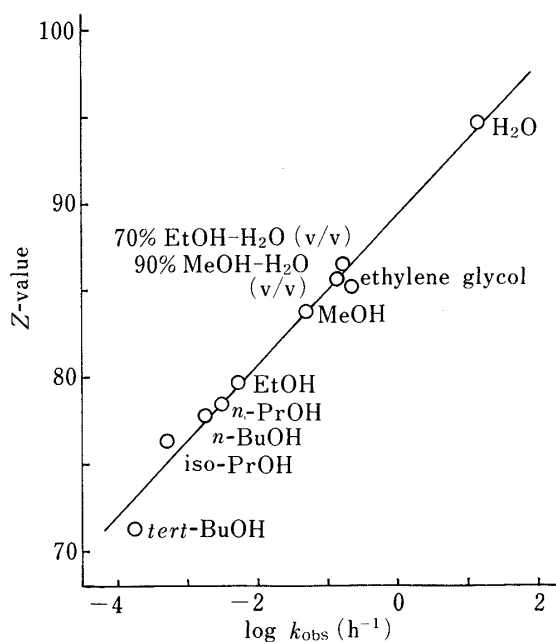


Fig. 1. Relation between k_{obs} and Z-Value

Apparent first-order rate constants obtained in a series of alcohols were plotted against Kosower's Z-values (corr. = 0.9908).

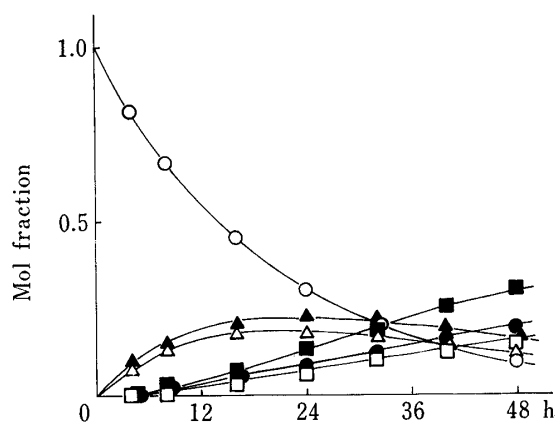


Fig. 3. Time Courses of I (○), II (△), III (□), IV (▲), V (■) and VI (●) during the Degradation of I in Methanol at 60°C

The lines are calculated values based on Chart 6 assuming $k_1=k_2=0.023\text{ h}^{-1}$, $k_3=0.027\text{ h}^{-1}$, $k_4=0.035\text{ h}^{-1}$, $k_5=0.022\text{ h}^{-1}$ and $k_6=0.005\text{ h}^{-1}$ by trial-and-error fitting (for equations, see ref. 3).

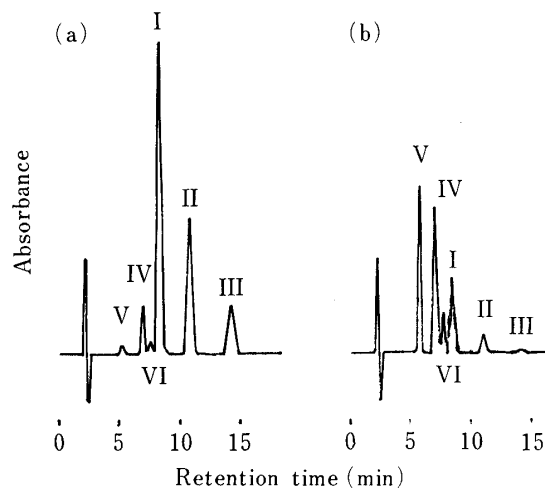


Fig. 2. Chromatograms Obtained from I Stored in Methanol at 60°C for 24 h by HPLC

(a) The wavelength of the UV monitor was set at 330 nm. (b) The wavelength of the UV monitor was set at 290 nm.

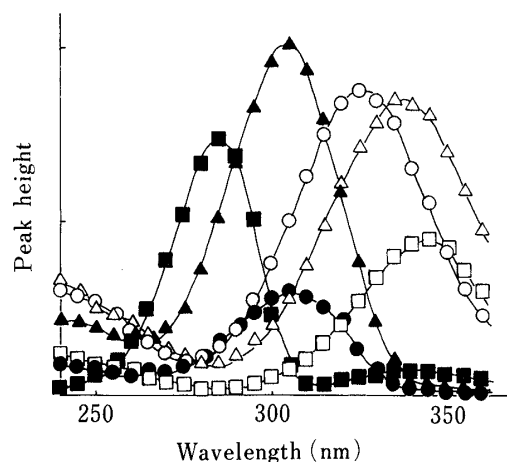


Fig. 4. UV Spectra of I (○), II (△), III (□), IV (▲), V (■) and VI (●)

I was stored in methanol at 60°C for 24 h, and the reaction mixture thus obtained was loaded onto a HPLC column. The wavelength of the UV monitor was changed from 240 to 360 nm by 5 nm in every loading. The peak heights of I—VI in chromatograms thus obtained were plotted against wavelength. The heights of I were plotted after multiplying by 0.5 to make the heights comparable to the others.

that the degradation mechanisms of I in alcohols may bear a close resemblance to those in aqueous solution.

Degradation Products of I in Methanol

To investigate the degradation mechanisms of I in alcohols, methanol was selected as a model alcohol, since the degradation rate of I is relatively high and it is suitable for detailed study. Typical HPLC patterns of I stored in methanol are shown in Fig. 2, where five peaks

are observed as degradation products. Their time courses (shown in Fig. 3) suggest that II and IV may be primary products, and III, V and VI may be secondary products. Ultraviolet (UV) spectra of all the peak components are shown together with that of I in Fig. 4. In comparison with the position of λ_{\max} of I, those of II and III exhibit red-shifts and those of IV, V and VI exhibit blue-shifts.

Compound I yields five degradation products in aqueous solutions of neutral pH, namely 5-(1-aziridinyl)-2-(2-hydroxyethyl)amino-1,4-benzoquinone, 2,5-di(2-hydroxyethyl)amino-1,4-benzoquinone, 5-(1-aziridinyl)-2-hydroxy-1,4-benzoquinone, 2,5-dihydroxy-1,4-benzoquinone and 5-hydroxy-2-(2-hydroxyethyl)amino-1,4-benzoquinone.³⁾ The UV spectra of these degradation products indicate that the hydrolysis of one aziridine ring causes a red-shift in λ_{\max} by about 10 nm, and the substitution of one aziridine ring by hydroxide causes a blue-shift in λ_{\max} by about 20 nm.¹³⁾ This result also suggests close similarity between the degradation of I in aqueous solution and that in methanol, and further suggests that II and III might be products of a kind of aziridine ring cleavage reaction caused by methanol (methanolysis), while IV, V and VI might be products of a kind of substitution reaction at the aziridine ring moiety (substitution by methylate).

The complex degradation process of I in aqueous solution becomes simplified by conducting the reaction either in acidic solution or in basic solution, *i.e.* in the former case, the hydrolytic cleavage of the aziridine ring alone is observed, and in the latter case, only the substitution of the aziridine ring by hydroxide is observed.³⁾ Similar behavior should be observed in the degradation of I in methanol, if the acidity and the basicity of methanol can be controlled. Therefore, the degradation of I was conducted in proton-rich methanol and methylate-rich methanol.

Degradation of I in Proton-Rich Methanol

Only II and III were observed as degradation products when I was stored in proton-rich methanol.¹⁴⁾ Their time courses indicate that the degradation follows the path I \rightarrow II \rightarrow III (Fig. 5).

As mentioned above, III may be a methanolysis product of the two aziridine rings of I, *i.e.* 2,5-di(2-methoxyethyl)amino-1,4-benzoquinone. This was confirmed by elemental analysis (Calcd for $C_{12}H_{18}N_2O_4$: C, 56.69; H, 7.09; N, 11.02. Found: C, 56.23; H, 6.98; N, 10.97) and the molecular peak in the mass spectrum (MS) ($M^+ = 254$) of isolated III. On the other hand, isolated II was degraded to III in proton-rich methanol at the same rate as I, when stored under the same conditions.

From these results, it is apparent that I is degraded to 2,5-di(2-methoxyethyl)amino-1,4-benzoquinone (III) *via* 5-(1-aziridinyl)-2-(2-methoxyethyl)amino-1,4-benzoquinone (II) by sequential methanolysis of the two aziridine rings (Chart 2). In this degradation process, the aziridine ring is protonated to yield the iminium ion, then the carbon at the 2' position of the

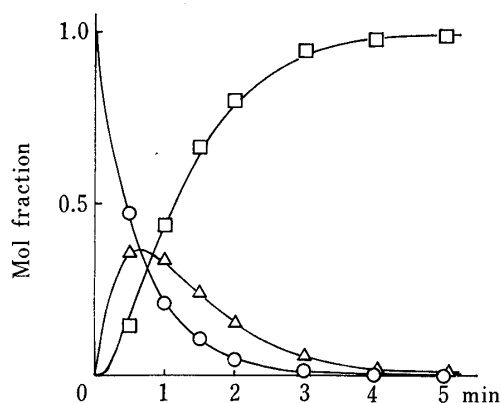


Fig. 5. Time Courses of I (O), II (Δ) and III (\square) during the Degradation of I in Proton-Rich Methanol at 60°C

The apparent proton concentration was adjusted to 10^{-4} M. The lines are calculated values based on Chart 2 assuming $k_1 = k_2 = 1.48 \text{ min}^{-1}$ by trial-and-error fitting (for equations, see ref. 3).

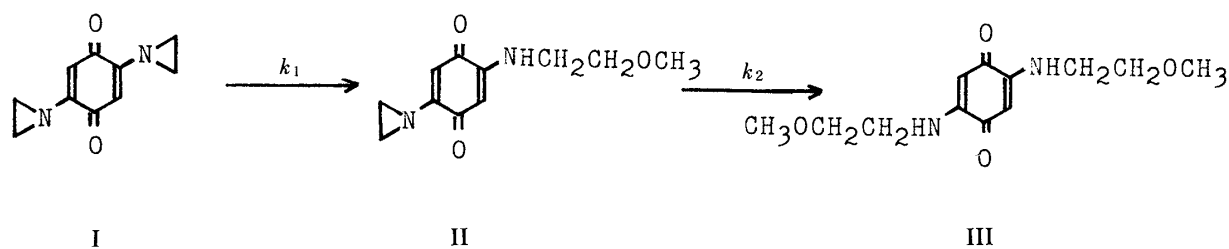


Chart 2

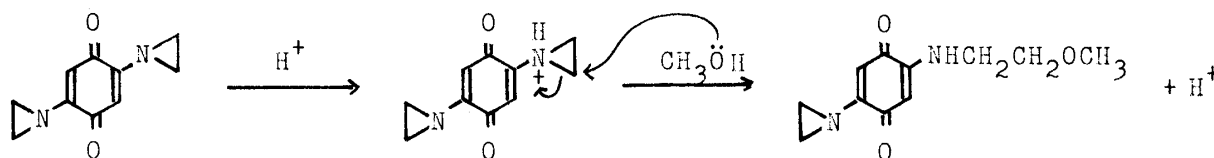


Chart 3

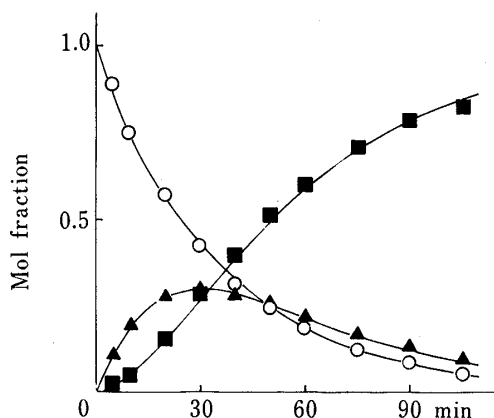


Fig. 6. Time Courses of I (○), IV (▲) and V (■) during the Degradation of I in Methylate-Rich Methanol

The apparent methylate concentration was adjusted to 10^{-3} M. The lines are calculated values based on Chart 4 assuming $k_3=0.028 \text{ min}^{-1}$ and $k_4=0.040 \text{ min}^{-1}$ by trial-and-error fitting (for equations, see ref. 3).

aziridine ring is exposed to the nucleophilic attack of methanol (Chart 3). This mechanism is essentially the same as that of I in acidic aqueous solution³⁾ although the nucleophile is methanol here instead of water.

Degradation of I in Methylate-Rich Methanol

Only IV and V were observed as degradation products when I was stored in methylate-rich methanol.¹⁵⁾ Their time courses indicate that the degradation follows the path I→IV→V (Fig. 6). Compound V was confirmed to be 2,5-dimethoxy-1,4-benzoquinone in comparison with the authentic compound. Isolated IV was degraded to V in methylate-rich methanol.

Therefore, in this system, it is apparent that I is degraded to 2,5-dimethoxy-1,4-benzoquinone (V) via 5-(1-aziridinyl)-2-methoxy-1,4-benzoquinone (IV) with sequential substitution of the two aziridine rings by methylate (Chart 4). In this degradation process, methylate attacks the carbon at the 2 position of benzoquinone as a nucleophile (Michael reaction),¹⁶⁾ then the aziridine ring is eliminated from the adduct (Chart 5). This mechanism is also essentially the same as that of I in basic aqueous solution³⁾ although the nucleophile is methylate here instead of hydroxide.

Degradation Process of I in Methanol

The studies described above revealed the chemical structures of four out of the five degradation products of I in methanol, *i.e.* II—V, and the mechanisms by which these products are formed. However, those of VI were not ascertained. Compound VI was formed

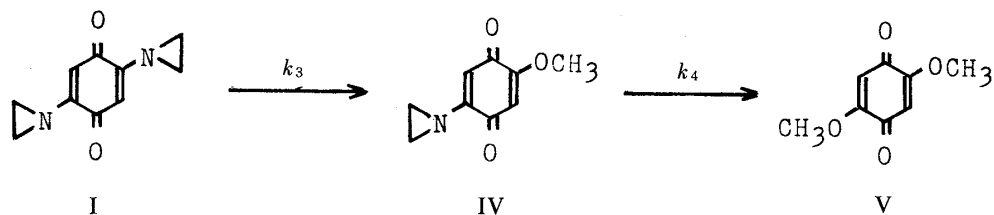


Chart 4

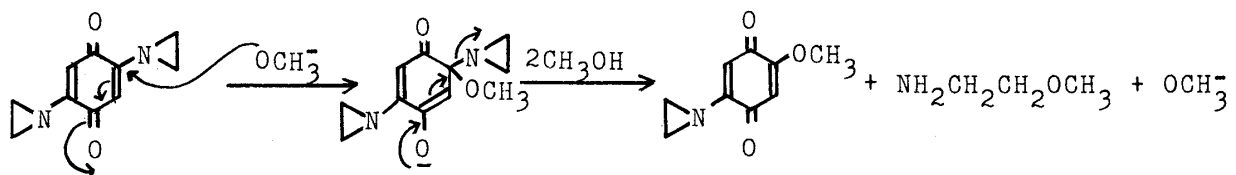


Chart 5

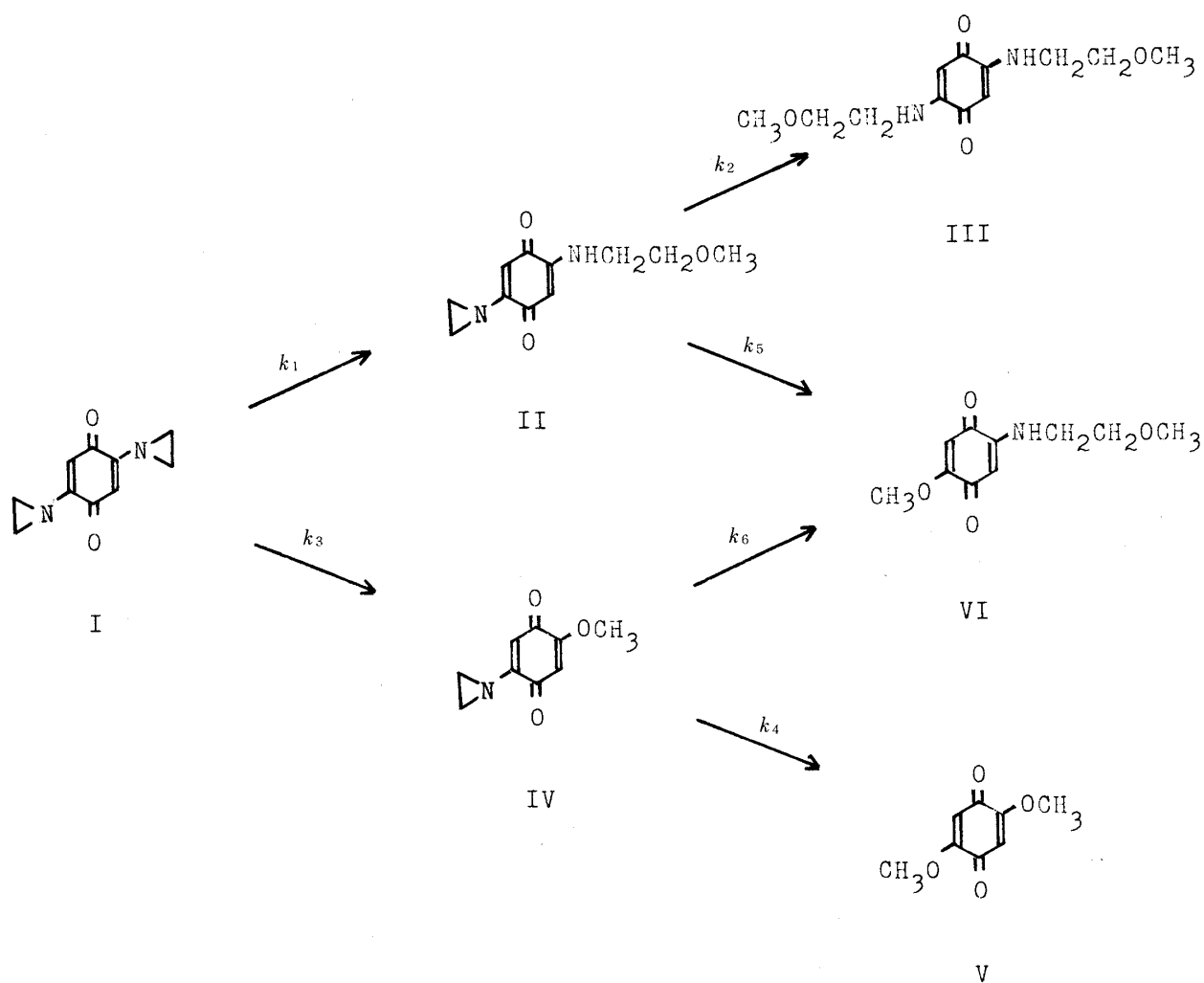


Chart 6

either by storing II in methoxide-rich methanol or by storing IV in proton-rich methanol. In methanol, II was degraded to III and VI, and IV were degraded to V and VI. From these results, VI is considered to be 5-methoxy-2-(2-methoxyethyl)amino-1,4-benzoquinone. Compound III was very stable in methanol, and the degradation of III was practically

unobservable.

By putting these results together, the degradation of I in methanol is concluded to proceed by a combination of methanolysis of the aziridine ring and substitution of the aziridine ring by methylate (Chart 6). Again, this is essentially the same as the degradation process of I in aqueous solutions at neutral pH.³⁾

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References and Notes

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- 7) F. Benington, R. D. Morin and L. C. Clark, Jr., *J. Org. Chem.*, **20**, 102 (1955).
- 8) DMA was used as a stock solvent for I, because I is practically inert in it, and its presence (~ 15%) does not affect the degradation rate of I in alcohols except for ethylene glycol (EG). In EG, the degradation rate shows some dependence on DMA concentration, but the rate constant obtained with 1% DMA was plotted in Fig. 1. This may be the reason why the rate constant for EG is a little off the line.
- 9) Precoated, layer thickness 0.25 mm (E. Merck, Darmstadt, Germany).
- 10) Prepacked column size A (240-10), LiChroprep RP-8 (40—63 μm) (E. Merck, Darmstadt, Germany).
- 11) ERC-ODS-1262 (Erma Optical Works, Ltd., Tokyo, Japan).
- 12) E. M. Kosower, *J. Am. Chem. Soc.*, **80**, 3253, 3261, 3267 (1958).
- 13) Unpublished data.
- 14) This was observed when the apparent proton concentration was higher than 10^{-5} M.
- 15) This was observed when the apparent methylate concentration was higher than 10^{-5} M.
- 16) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," 2nd ed., Cornell University Press, Ithaca, 1969, pp. 1015—1023.