# A Novel Redox Reaction of Ebselen with Alcohol in Basic Solution

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**Ebselen is an organoselenium compound that exhibits a glutathione peroxidase-like activity. The kinetics of ebselen degradation in basic aqueous solution containing primary alcohol was investigated by HPLC. The reversible reaction of ebselen and its dimer (diselenide) was discovered as a novel redox reaction. This reaction followed first-order degradation in basic solution at a constant temperature, and the equilibrium constants of ebselen and diselenide depended on pH. Analysis of the degradation rate for the forward reaction-pH profile indicated that specific base catalysis occurs in ebselen. The mechanism of this redox reaction was investigated by HPLC, spectrophotometry and trapping the labile intermediate with trichloroethylene. Ebselen decomposes to diselenide *via* selenenic acid and selenol by reacting with primary alcohol. The deuterium isotope effect on the forward reaction indicated that the reaction rate depends on the strength of the hydrogen–carbon bond at the α-position of primary alcohol. On the other hand, the effect of nitrogen replacement on the reverse reaction showed that diselenide is converted into ebselen by oxidation with oxygen existing in the solution.**

**Key words** ebselen; degradation; redox reaction

Ebselen [2-phenyl-1,2-benzisoselenazol-3(2*H*)-one] (**1**, Chart 1) is an organo-selenium compound which exhibits glutathione peroxidase-like activity by catalyzing the reduction of $H_2O_2$ and other hydroperoxides *in vitro*.[1,2] In addition, a glutathione-independent antioxidant capacity was found during microsomal lipid peroxidation,[1] similar to that observed with diethyldithiocarbamate.[3] Ebselen was also able to inhibit ADP-Fe-induced lipid peroxidation in isolated hepatocytes, dependent on the presence of intracellular glutathione.[3] This compound is being developed as an effective medicine for the treatment of cerebral infarction and subarachnoid hemorrhage.

Generally, the stability of a drug is an important factor in planning the transport, storage and manufacturing process of preparations. Stability data also form an essential part of the documents in a new drug application. However, the stability of organic selenium compounds are not well known. Thus, the stability of ebselen and its degradation under severe conditions were studied. Ebselen is stable in acidic solution but unstable in basic solution. This study describes in detail the stability of ebselen under highly stressed conditions in basic solution. Further, we investigated the pathway, kinetics and mechanism of the degradation reaction of ebselen.

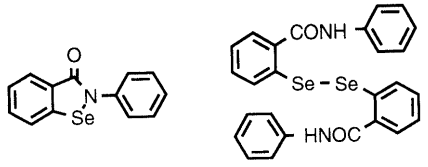
## Experimental

**Materials** Ebselen and 2,2′-diseleno-bis(benzanilide) (diselenide, **2**) were from Rhône-Poulenc Nattermann (Cologne, Germany). Ethyl-1,1-$d_2$ alcohol ([1,1-$D_2$]-EtOH) was from Aldrich Chem. Co. (Milwaukee, U.S.A.). All solvents and reagents used were of analytical or reagent grade. The water used was purified with a Milli-Q system (Millipore Co., Ltd., Massachusetts, U.S.A.).

**Procedure for the Degradation of Ebselen and Diselenide** Ebselen and **2** were dissolved in a mixture of 60% aqueous solution and 40% alcohol as follows. In an experiment on the kinetics of degradation, the mixtures of 60% Britton–Robinson buffer and 40% methanol or 60% aqueous solution containing NaOH and 40% methanol were used as solvents. The pH values of Britton–Robinson buffer used were pH 11.0, pH 11.5 and pH 12.0, and the concentrations of NaOH used were 0.018 N, 0.03 N and 0.1 N. In studying the effects of alcohols, mixtures of 60% aqueous solution containing NaOH and 40% alcohol were used as the solvent, and ethanol (EtOH), isopropyl alcohol (iso-PrOH), *tert*-butylalcohol (*t*-BuOH), [1,1-$D_2$]-EtOH were used as the alcohols. The concentrations of NaOH were prepared so that the pH values of the samples were *ca.* pH 13.0. For studying the effects of oxygen, samples of ebselen and **2** in 60% 0.018 N NaOH and 40% methanol were bubbled with nitrogen gas in order to remove dissolved oxygen. The concentrations of ebselen and **2** were *ca.* 40 μg/ml and *ca.* 20 μg/ml, respectively. These samples (1.0ml) were kept at 25, 60, 70 or 80 °C in capped tubes and cooled on ice to stop the reaction at suitable time intervals. For the determination of ebselen alone, except for the study of the effects of alcohols, 50 μl of a solution of 2,6-xylidine in methanol (0.1 v/v%) was added to the sample as an internal standard. For simultaneous determination of ebselen and **2**, 100 μl of a solution of 2,4,5-trichloroaniline, an internal standard, in methanol (200 μg/ml), and dimethyl sulfoxide (3.0 ml) were added to the sample. To study the effects of alcohols, the samples were analyzed to determine ebselen without any additives.

**HPLC Analysis of Ebselen and Diselenide** HPLC analysis of ebselen and **2** were carried out using the Shimadzu LC-10A system (Shimadzu Co., Kyoto). Samples of 20 μl were injected onto a reverse phase column (Inertsil ODS-2 column 15 × 0.4 cm i.d., GL Sciences, Inc.). Ebselen and **2** were monitored by UV absorption at 254 nm. The mobile phase consisted of mixtures of a 50 mM phosphate buffer of pH 4.6 (A) and acetonitrile (B), and the flow rate was 1.0 ml/min at 40 °C. A mixture of 65% A and 35% B was used to elute ebselen (retention time ($t_R$), 11.5 min); a mixture of 40% A and 60% B was used for ebselen and **2** ($t_R$ of ebselen, 3.3 min; $t_R$ of **2**, 11.2 min).

**Isolation of Diselenide** Ebselen (40 mg) was dissolved in the mixture of 60% 0.1 N NaOH and 40% methanol so that the total volume was 1 l. This sample was kept at 80 °C for 2 h. The reaction solution was subjected to HPLC in order to confirm the reaction. The HPLC analysis used was the same method used for the simultaneous determination of ebselen and **2** as described above. After reaction, the precipitate, **2**, was separated from the solution by filtration, washed with water, and dried under vacuum. $^1$H-NMR and $^{13}$C-NMR spectra of isolated **2** and the authentic sample of **2** in dimethyl sulfoxide-$d_6$ was measured at 25 °C with a JEOL JNM-GSX500 NMR spectrometer (JEOL, Ltd.). Tetramethylsilane was used as an internal standard. FDMS of isolated




Ebselen(**1**) Diselenide(**2**)

Chart 1

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**2** and the authentic sample of **2** were measured with a JEOL JMS-110 MS spectrometer (JEOL, Ltd.). IR spectrum of isolated **2** and the authentic sample of **2** were measured with a JEOL JIR-5300 FT-IR spectrometer (JEOL, Ltd.).

**Spectral Measurements of Ebselen** The absorption spectra of ebselen were measured using a U-3300 spectrophotometer (Hitachi, Ltd.). The concentration of ebselen was *ca.* 10 $\mu$g/ml. Solvents used were mixtures of 60% 1.0 N HCl and 40% methanol, 60% pH 7.0 Britton–Robinson buffer and 40% methanol, or 60% 1.0 N NaOH and 40% methanol. The pH values of the samples were 0.4, 7.9 and 13.6, respectively. As for the sample of 60% 1.0 N NaOH and 40% methanol, the pH value of the sample was adjusted to 1.5 after measurement of the spectrum. Then, the absorption spectrum of the sample was immediately measured again. $^{77}$Se-NMR spectra were measured at 25 °C using a JEOL JNM-GSX500 NMR spectrometer. The concentration of ebselen was *ca.* 1 mg/ml. $Me_2Se$ was used as an external standard. Solvents used were mixtures of 30% 0.1 N DCl and 70% methanol-$d_4$, and 30% 0.1 N NaOD and 70% methanol-$d_4$.

**Preparation of 3** Trichloroethylene (0.8 ml) was diluted by methanol so that the total volume was 8.0 ml. This solution was added to the solution of ebselen in a mixture of 60% 0.1 N NaOH and 40% methanol. The concentration of ebselen was *ca.* 125 $\mu$g/ml. This sample was kept at 90 °C for 3.5 h. The reaction solution was subjected to HPLC in order to confirm the reaction. The same HPLC analysis method was used for the simultaneous determination of ebselen and **2** as described above. After the reaction, the precipitate, **3**, was separated from the solution by filtration, washed with water, and dried under vacuum. The $^1$H-NMR and $^{13}$C-NMR spectra of **3** in dimethyl sulfoxide-$d_6$ were measured at 25 °C using a JEOL JNM-GSX500 NMR spectrometer. Tetramethylsilane was used as an internal standard. Electron impact-mass spectra (EIMS), field desorption-mass spectrum (FDMS) and fast atom bombardment mass spectrometry (FABMS) of **3** were measured using a JEOL JMS-110 MS spectrometer.

## Results and Discussion

**Kinetics of Degradation Reaction** A basic aqueous solution containing methanol was used as the solvent in the degradation reaction in order to raise the solubility of ebselen in this study. Compound **2** was detected as only one major degradation product of ebselen in basic solution by HPLC (Fig. 1). Since the concentration of ebselen came to a constant value in the degradation reaction, it was suggested that ebselen was in equilibrium with **2**. Table 1 lists the results of the assay of ebselen and **2**, as well as the sum of their concentrations in the degradation reaction of ebselen or **2**. The total concentrations of ebselen and **2** were above 87% in both degradation reactions. These findings indicated that the main degradation product of ebselen was **2**, whereas the main degradation product of **2** was ebselen. The degradation product, **2**, was isolated and identified by a comparison of its $^1$H-NMR spectrum, $^{13}$C-NMR spectrum, IR spectrum and FDMS spectrum with those of authentic **2**. Moreover, the concentration ratio of ebselen and **2** in the sample of ebselen degradation became similar to that in the sample of **2** degradation as the reaction time became longer. Therefore, ebselen was confirmed to be in equilibrium with **2**. Reich and Jasperse have reported that isoselenazolidin-3-one or selenenic acid undergo disproportionation.[4,5] However, a reversible reaction had not yet been reported in this category of compounds. Thus, the reaction studied in this report was shown to be the first example of a reversible reaction between ebselen and **2**.

Since the degradation reaction of ebselen was reductive dimerization, ebselen could be considered to degrade following second-order kinetics. However, the reaction

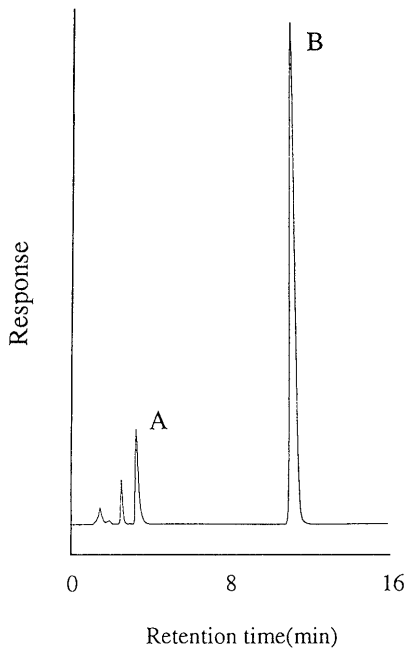



Fig. 1. Chromatogram of Ebselen and Its Degradation Product (A), ebselen; (B), diselenide, **2**.

Table 1. Relative Molar Concentrations of Ebselen and Diselenide (**2**) in the Degradation Reaction of Ebselen and Diselenide at 80 °C and pH 12.2

| Time (h) | Ebselen (%) | Diselenide (%)[a] | Total value (%) |
|---|---|---|---|
| Ebselen | | | |
| 0 | 100 | 0 | 100 |
| 1 | 81.7 | 17.9 | 99.6 |
| 2 | 70.9 | 25.6 | 96.5 |
| 4 | 61.7 | 30.9 | 92.6 |
| 6 | 58.2 | 33.1 | 91.3 |
| 8 | 55.6 | 34.8 | 90.4 |
| Diselenide | | | |
| 0 | 0 | 100 | 100 |
| 2 | 44.4 | 47.8 | 92.2 |
| 4 | 53.5 | 36.0 | 89.5 |
| 8 | 49.6 | 37.7 | 87.3 |

a) The relative molar concentrations of diselenide are doubled, because it is formed by dimerization.

rate was found to follow good first-order kinetics. Based on the reversible reaction of ebselen (**1**) and diselenide (**2**), the following equations can be derived:

$$-d[A]/dt = k_1[A] - 2k_{-1}[B] \quad (1)$$

$$[A]_0 = [A] + 2[B] = [A]_e + 2[B]_e \quad (2)$$

$$k_1/k_{-1} = 2[B]_e/[A]_e \quad (3)$$

where $k_1$ and $k_{-1}$ indicate the first-order rate constants for the forward reaction and the reverse reaction, respectively, [A] and [B] indicate the concentrations of ebselen and diselenide, respectively, $[A]_0$ and $[A]_e$ indicate the concentrations of ebselen at the initial time and at equilibrium respectively, and $[B]_e$ indicates the concentration of diselenide at equilibrium.

Combination of Eq. 1, Eq. 2 and Eq. 3 gives:

$$-d[A]/dt = (k_1 + k_{-1})([A] - [A]_e) \quad (4)$$

Table 2. Degradation Rate Constants of Ebselen and Equilibrium Constants ($K$)

| pH | $k_1$ ($h^{-1}$) | | | $k_{-1}$ ($h^{-1}$) | | | $K (=k_1/k_{-1})$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | 60 °C | 70 °C | 80 °C | 60 °C | 70 °C | 80 °C | 60 °C | 70 °C | 80 °C |
| 11.3 | — | — | 0.05 | — | — | 0.31 | — | — | 0.16 |
| 11.7 | — | — | 0.11 | — | — | 0.32 | — | — | 0.34 |
| 11.9 | 0.02 | 0.07 | 0.17 | 0.04 | 0.15 | 0.31 | 0.50 | 0.47 | 0.55 |
| 12.2 | — | — | 0.29 | — | — | 0.29 | — | — | 1.00 |
| 12.4 | — | — | 0.50 | — | — | 0.23 | — | — | 2.17 |
| 13.0 | — | — | 1.85 | — | — | N.D. | — | — | N.D. |

N.D., not detected.

The integral of Eq. 4 gives Eq. 5, and the first-order rate constants, $k_1$ and $k_{-1}$, could be obtained from Eq. 5, Eq. 6 and Eq. 7.

$$[A]_t=[A]_e+([A]_0-[A]_e)\exp(-k_{obs}\cdot t) \quad (5)$$

$$k_{obs}=k_1+k_{-1} \quad (6)$$

$$k_1/k_{-1}=([A]_0-[A]_e)/[A]_e \quad (7)$$

where $[A]_t$ indicates the concentration of ebselen at time $t$. The kinetic parameters, $k_{obs}$ and $[A]_e$, were calculated from Eq. 5 by the non-linear least squares fit method. Then, $k_1$ and $k_{-1}$ were calculated from Eq. 6 and Eq. 7 by $k_{obs}$ and $[A]_e$. We precluded the influence of an unknown degradation product on this calculation, because the unknown degradation product was in small quantities. In Fig. 2, the lines show the theoretical curves obtained, while the points represent experimental results. The reasonable degree of agreement between the theoretical and experimental values indicates that Eq. 5 adequately describes the kinetics of the degradation reaction, and an intermediate exists in the forward reaction as described below. The rate constants and equilibrium constants obtained are shown in Table 2. If this reaction is catalyzed by the hydroxide ion, $k_1$ is represented by Eq. 8.

$$k_1=k_{OH}\cdot a_{OH} \quad (8)$$

where $k_{OH}$ and $a_{OH}$ are the rate constant of specific base catalysis and the hydroxyl ion activity, respectively. The plots of $\log k_1$ *versus* pH (not shown) gave a good linear relationship with a correlation coefficient $>0.99$. This result confirmed that ebselen degrades by the mechanism of specific base catalysis.

For studying the temperature dependency of the degradation reaction, the rate constants ($k_1$) and absolute temperatures ($T$) were fitted by the method of least squares to the Arrhenius expression of Eq. 9.[6]

$$k_1=A\cdot\exp(-E_a/RT) \quad (9)$$

where $E_a$ is the activation energy and $A$ is the frequency factor. The plots of $\ln k_1$ *versus* $1/T$ (not shown) gave a good linear relationship with a correlation coefficient $>0.98$. This result confirmed that the rate of the degradation of ebselen depends on temperature. On the other hand, the rate constant for the reverse reaction, $k_{-1}$, remained constant at various pH values tested. Thus, in the range of pH $>11$, the equilibrium constant increased extremely as the pH increased. The equilibrium constant depended on pH value, but it was independent of temperature.

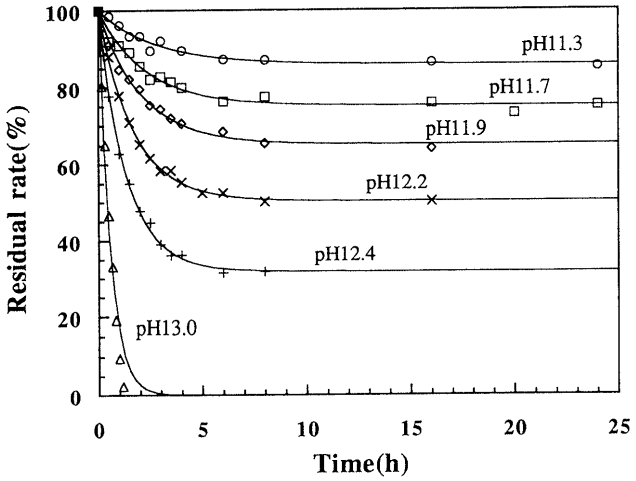



Fig. 2. Residual Rate of Ebselen in Solutions at Various pHs at 80 °C
Points: experimental, lines: theoretical curve.

**Mechanism of Degradation Reaction** First, the mechanism of the reverse reaction was investigated. Since **2** must be oxidized to generate ebselen, the effects of oxygen existing in the solution was studied (Fig. 3). The decomposition of ebselen was accelerated by nitrogen substitution, compared with the decomposition of ebselen under air. On the other hand, the formation of ebselen from **2** was inhibited by nitrogen substitution. Therefore, **2** was considered to generate ebselen by oxidation with the oxygen dissolved in the solution. It has been reported that **2** reacts with hydrogen peroxide and ebselen is generated *via* selenenic acid anhydride as an intermediate.[7] Consequently, diselenide reacts with dissolved oxygen, and then ebselen is considered to be formed *via* selenenic acid anhydride.

Next, the mechanism of the forward reaction was investigated. The degradation rate of ebselen was measured in basic solution containing various alcohols by the HPLC method (Table 3). The order of degradation rate was iso-PrOH > EtOH > MeOH and no degradation in the solution containing *t*-BuOH could be detected. The degradation rate was considered to depend on the ease of cleavage of the hydrogen–carbon bond at the α-position of alcohol because the order of the degradation rate follows the oxidative potential of alcohol,[8] and no degradation in the solution containing *t*-BuOH was confirmed. For the confirmation, we investigated the isotope effect in the degradation rate using [1,1-$D_2$]-EtOH. Ebselen degraded

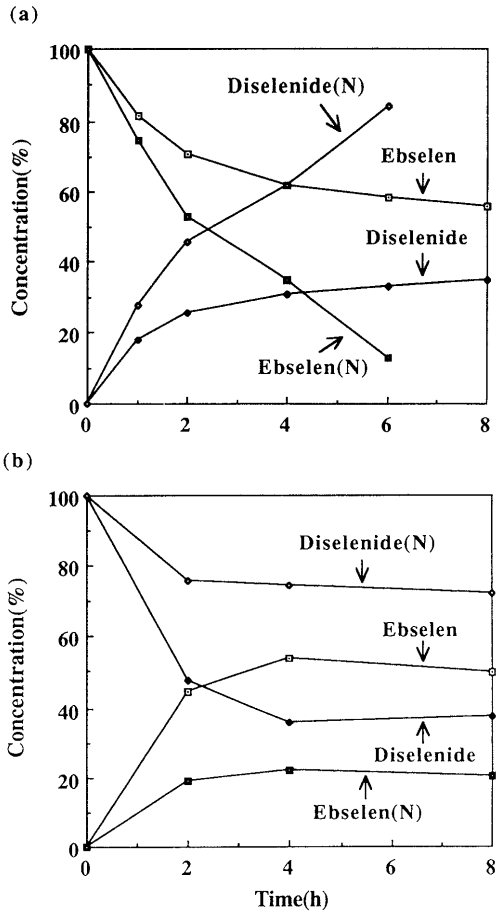



Fig. 3. Relative Molar Concentrations of Ebselen and Diselenide, **2**, in the Degradation Reaction of Ebselen (a) and Diselenide (b) at 80 °C and pH 12.2

N: Under nitrogen. The relative molar concentrations of diselenide are doubled, because it is formed by dimerization.

Table 3. Effects of Alcohols on the Degradation Rate of Ebselen

| Temp. (°C) | Time (h) | Ebselen (%) | | | |
|---|---|---|---|---|---|
| | | MeOH pH 13.0 | EtOH pH 12.9 | iso-PrOH pH 13.0 | *t*-BuOH pH 12.9 |
| 25 | 0 | 100 | 100 | 100 | 100 |
| | 0.5 | — | — | 14.0 | — |
| | 2 | 97.2 | 67.2 | — | 99.0 |
| | 4 | 98.4 | 41.7 | — | 97.7 |
| 80 | 0 | 100 | — | — | 100 |
| | 20 min | 61.3 | — | — | 98.9 |
| | 50 min | 22.3 | — | — | 97.9 |

Results are shown as relative molar concentrations of ebselen in 40% alcohols/60% aqueous solution containing NaOH.

following first-order kinetics in both solutions containing EtOH and [1,1-$D_2$]-EtOH (Fig. 4). The ratio of the degradation rates of the EtOH system and [1,1-$D_2$]-EtOH system is 6.2. This isotope effect indicates that the reaction includes fission of the hydrogen–carbon bond at the α-position of alcohol and resulted in the oxidation of the alcohols.

The absorption spectra of ebselen were measured in acidic, neutral and basic solutions. The spectrum patterns of ebselen in acidic and neutral solution were similar, whereas that in basic solution was different from the other

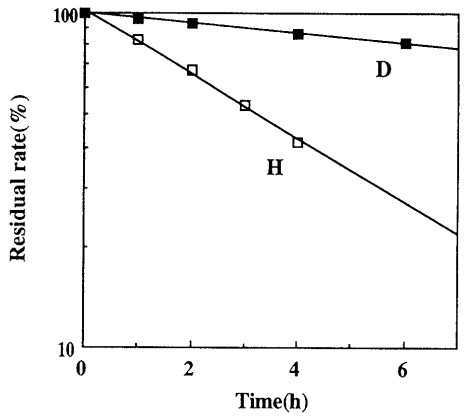



Fig. 4. Isotope Effect on the Degradation Rate of Ebselen

Residual rate of ebselen in 40% EtOH or [1,1-$D_2$]-EtOH/60% aqueous solution containing NaOH at 25 °C and pH 12.9 are shown. H: EtOH; D: [1,1-$D_2$]-EtOH.

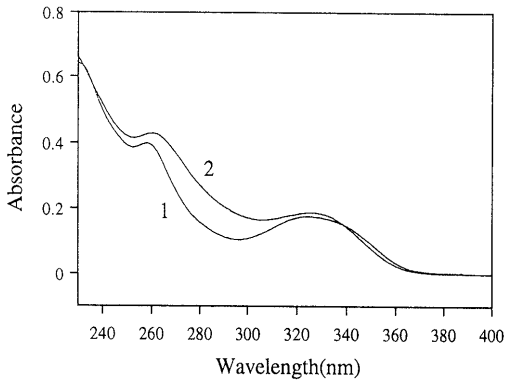
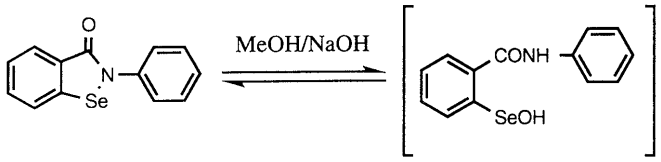



Fig. 5. Absorption Spectra of Ebselen at Various pHs

Solvent: 1, 40% MeOH/60% 1.0 N HCl; 2, 40% MeOH/60% 1.0 N NaOH, similar spectrum pattern to 1 was obtained in 40% MeOH/60% pH 7.0 buffer. Concentration: 10 μg/ml.




**Ebselen** **(4)**

Chart 2

two spectrum patterns (Fig. 5). Moreover, the absorption spectrum of the basic solution of ebselen, after the pH was adjusted to 1.5, was almost the same as that of ebselen in acidic and neutral solutions. These findings suggested that ebselen formed a different species in basic solution, and this reaction was reversible. This speculation was also supported by the broad signal of ebselen in basic solution in the $^{77}$Se-NMR spectrum. The Se–N bond of selenenamide was reported to be hydrolyzed in water.[9] Consequently, it was speculated that the species formed under a basic condition was selenenic acid (**4**) and it was in equilibrium with ebselen (Chart 2).

The selenenic acid, represented as RSeOH, was reported to be active and to add to olefins easily.[10] So, ebselen was allowed to react with trichloroethylene in a basic solution containing methanol to trap the intermediate in the degradation reaction of ebselen to **2**. The formation

of **2** was not detected in the sample, whereas ebselen reacted with trichloroethylene and formed **3** selectively. The chemical structure of **3** was confirmed by comparing the $^1$H-NMR spectrum, $^{13}$C-NMR spectrum and MS spectra of **3** with those of ebselen (Table 4). In addition to the proton signals observed in the spectrum of ebselen, signals of an olefinic proton and amide proton were observed at 7.5 ppm and 10.5 ppm, respectively, in the $^1$H-NMR spectrum of **3**. Two additional carbon signals were also observed at 124 ppm and 128 ppm in the $^{13}$C-NMR spectrum of **3**. The carbon signal at 128 ppm showed a cross peak with the olefinic proton signal at 7.5 ppm. Ion peaks were observed at *m/z* 371 in EIMS and FDMS, and at *m/z* 372 in FABMS. These findings support the proposed chemical structure of **3**, shown in Chart 3, in which the Se–N bond in ebselen was hydrolyzed and the selenium atom replaced the chlorine atom on the C-2 carbon of trichloroethylene. If the selenium atom was on the C-1 carbon, two geometrical stereo isomers could have resulted. However, **3** showed a single olefinic proton signal in the NMR spectrum and a single peak in HPLC, with no evidence of the occurrence of two isomers.

The formation of **3** could not be explained by the addition of **4** to trichloroethylene, and the unusual elimination of HOCl. Thus, another intermediate, RSeX (R: the same moiety as in **4**, X: unknown functional group), must be responsible for the formation of **3**. Since the elimination of XCl was a very smooth reaction, X should be an electron releasing group. Considering that the degradation of ebselen was accompanied by the oxidation of alcohols in the solvent, it is quite probable that X was a hydrogen atom and the second intermediate was selenol (**5**).

Consequently, the mechanism of the degradation reaction from ebselen to **2** was considered to be as shown in Chart 4. Namely, ebselen was considered to decompose to **2** *via* **4** and **5**.

To obtain a more comprehensive view of the reaction, we investigated the mechanism by kinetics analysis. Since **4** and **5** were thought to be active intermediates, these species were considered to remain in the steady state condition for a short time. The derivation of the equations is shown below according to the mechanism depicted in Chart 4.

The reactions are:

Table 4. $^1$H-NMR and $^{13}$C-NMR Spectral Data of **3** in DMSO-$d_6$

| $^1$H-NMR | | $^{13}$C-NMR | |
|---|---|---|---|
| Proton No. | $\delta_{ppm}$ (mult, $J$=Hz) | Carbon No. | $\delta_{ppm}$ |
| 2 | 10.56 (s) | 3 | 165.95 |
| 4 | 8.02 (d, 8.0) | 4 | 129.03 |
| 5 | 7.48 (t, 8.0) | 5 | 126.51 |
| 6 | 7.58 (t, 8.0) | 6 | 132.00 |
| 7 | 7.45 (d, 8.0) | 7 | 129.68 |
| 10 | 7.53 (s) | 8 | 131.35 |
| 2′,6′ | 7.73 (d, 7.5) | 9 | 133.54 |
| 3′,5′ | 7.38 (t, 7.5) | 10,3′,5′ | 128.57, 128.62 |
| 4′ | 7.15 (t, 7.5) | 11, 4′ | 124.06, 124.09 |
| | | 1′ | 138.46 |
| | | 2′,6′ | 120.46 |

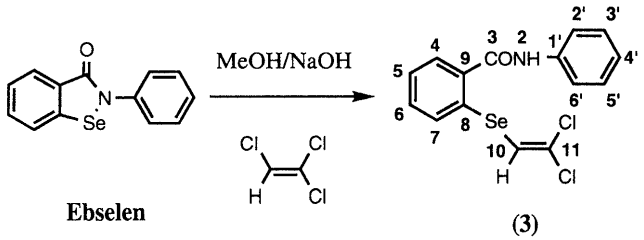



Chart 3

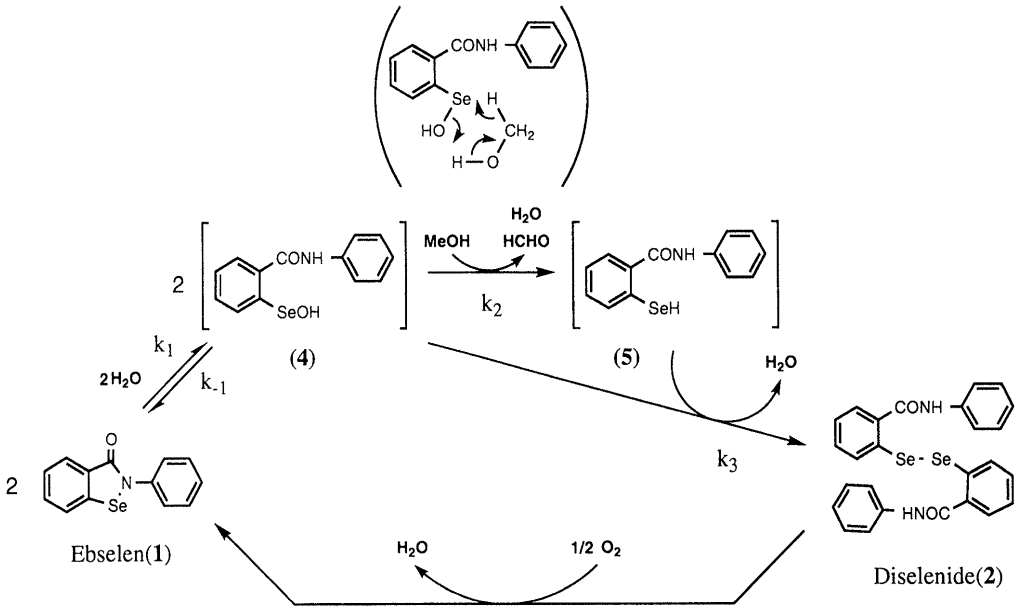



Chart 4

$$\text{ebselen} + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathbf{4} \quad \text{(I)}$$

$$\mathbf{4} + \text{MeOH} \xrightarrow{k_2} \mathbf{5} + \text{HCHO} + H_2O \quad \text{(II)}$$

$$\mathbf{4} + \mathbf{5} \xrightarrow{k_3} \mathbf{2} + H_2O \quad \text{(III)}$$

Based on this reaction scheme, the following equations can be derived:

$$-d[\text{ebselen}]/dt = k_1[\text{ebselen}] - k_{-1}[\mathbf{4}] \quad (10)$$

$$d[\mathbf{4}]/dt = k_1[\text{ebselen}] - k_{-1}[\mathbf{4}] - k_2[\mathbf{4}] - k_3[\mathbf{4}][\mathbf{5}] \quad (11)$$

$$d[\mathbf{5}]/dt = k_2[\mathbf{4}] - k_3[\mathbf{4}][\mathbf{5}] \quad (12)$$

In steady state conditions:

$$d[\mathbf{4}]/dt = k_1[\text{ebselen}] - k_{-1}[\mathbf{4}] - k_2[\mathbf{4}] - k_3[\mathbf{4}][\mathbf{5}] = 0 \quad (13)$$

$$d[\mathbf{5}]/dt = k_2[\mathbf{4}] - k_3[\mathbf{4}][\mathbf{5}] = 0 \quad (14)$$

Combination of Eq. 13 and Eq. 14 gives:

$$[\mathbf{4}] = k_1/(k_{-1} + 2k_2)[\text{ebselen}] \quad (15)$$

Combination of Eq. 10 and Eq. 15 gives:

$$-d[\text{ebselen}]/dt = 2k_1k_2/(k_{-1} + 2k_2)[\text{ebselen}] \quad (16)$$

Equation 16 shows that the degradation rate follows first-order kinetics and to what extent the alcohols affect the degradation rate. This equation is in good agreement with the experimental data of the degradation of ebselen.

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