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Degradation kinetics of KE-298 and chelating ability of its degradation products

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Abstract

The degradation kinetics of 2-acetylthiomethyl-3-(4-methylbenzoyl)propionic acid (KE-298; **1**) in aqueous solution were studied. Compound **1** followed pseudo-first-order degradation kinetics at constant pH and temperature, and yielded four types of degradation products. Analysis of the degradation rate-pH profile suggested that specific acid catalysis of unionized and ionized species, water catalysis of ionized species and specific base catalysis of ionized species occur for **1**. The degradation products of **1** were identified by HPLC and LC-MS, and the degradation pathways of **1** were clarified. Since 2-mercaptomethyl-3-(4-methylbenzoyl)propionic acid (KE-758; **2**), one of the degradation products of **1**, has chelating ability, the degradation pathway of **1** in aqueous solution was altered by the addition of certain metal ions, e.g., copper(II) ion, to the reaction solution.

Keywords: 2-Acetylthiomethyl-3-(4-methylbenzoyl)propionic acid; Degradation kinetics; Degradation product; Chelation; LC-MS; HPLC

1. Introduction

2-Acetylthiomethyl-3-(4-methylbenzoyl)propionic acid (KE-298; **1**) is an orally active antirheumatic agent synthesized by Kameo et al. (1988), and the structure-activity of **1** and its relative compounds has been studied by Kawashima et al. (1992). Compound **1** is very stable in the solid state but unstable in aqueous solution. Generally, the stability of a drug has important effects on the transport and process of manufacture of preparations as well as the results of toxicological, pharmacological and pharmacokinetics tests preformed during development. This study was performed to determine the general pattern of degradation of **1** under highly stressed conditions in aqueous solution.

In this study, 2-mercaptomethyl-3-(4-methylbenzoyl)propionic acid (KE-758; **2**), a principal metabolite of **1** in humans and animals, was detected as the main degradation product in aqueous solution. This compound has an SH functional group and displays two types of equilibrium conformations, and therefore appeared likely to possess chelating ability, as previously reported for D-penicillamine by Sugiura and Tanaka (1970,

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1972) and Wright and Frieden (1975). The chelating ability of D-penicillamine is closely related to its pharmacological properties, since Walsh (1953, 1956) reported that formation of a chelating compound with copper(II) and elimination of this compound were the mechanisms by which D-penicillamine exerts its therapeutic effects in this treatment of Wilson's disease. Therefore, if **2** has chelating ability, **1** can be expected to show the same kind of efficacy in treatment of Wilson's disease. Consequently, in order to determine whether or not **2** has chelating ability, stability studies in aqueous solutions were also performed.

2. Experimental

2.1. Materials and reagents

Compound **1** and authentic degradation compounds were synthesized at Taisho Pharmaceutical Co., Ltd. (Saitama, Japan). All solvents and reagents used were of analytical or reagent grade. The deionized water used in all experiments was obtained from a Mill-Q system (Waters, Milford, MA).

2.2. Buffer solutions

The following buffer solutions were used: hydrochloric acid (pH 1), sodium phosphate buffer (pH 2, 3, 6, 7, 7.4, 10), sodium acetate buffer (pH 4, 5), and sodium borate buffer (pH 8, 9). These buffer solutions were 0.1 M for hydrochloride, phosphate, acetate and borate, respectively, and were adjusted to a total ionic strength of 0.5 by the addition of sodium chloride.

2.3. High-performance liquid chromatography system

An HPLC system consisting of a constant-flow pump (Model 880-PU, Jasco, Tokyo, Japan) operated at 1 ml/min, a reversed-phase column packed with 5 μm TSK gel ODS 80TM (4.0 mm i.d. $\times$ 150 mm, 50° C, Tosoh, Tokyo, Japan), a variable-wavelength UV detector (Model 880-UV, Jasco) operated at 254 nm and an electronic integrator (C-R4A, Shimadzu, Kyoto, Japan) was used for all analyses. The mobile phase was aqueous 40% acetonitrile (v/v) containing 0.1% phosphoric acid.

2.4. Liquid chromatography / atmospheric pressure chemical ionization mass spectrometry system (LC /APCI-MS)

The APCI-MS system used was a Model M-1000 (Hitachi, Tokyo, Japan). The HPLC system used consisted of a constant-flow pump (Model L-6200, Hitachi) operated at 1 ml/min and a reversed-phase column packed with 5 μm TSK gel ODS 80TM (4.0 mm i.d. $\times$ 150 mm, 50° C, Tosoh, Tokyo, Japan). The mobile phase was aqueous 40% acetonitrile (v/v) containing 0.05% trifluoroacetic acid. The drift, focus and multiplier voltages of the APCI interface were set at 120 V, 20 V and 2 kV, respectively. The temperatures of the vaporizer and desolvation region of the APCI interface were set at 265 and 399° C, respectively. The scan range and speed of the MS spectrometer were 5–500 and 500 a.m.u. per 2 s, respectively.

2.5. Kinetic procedures

2.5.1. pH-rate profile

A stock solution of **1** (10 mg/ml) was prepared in ethanol in an amber volumetric flask. Aliquots taken from the stock solution were diluted with the reaction buffer to give a final concentration of 0.1 mg/ml. All sample solutions were sealed in clear glass ampules. Degradation was carried out in a constant temperature oil bath at 60° C with shielding from light. The reaction samples were withdrawn at suitable time intervals, cooled on an ice bath, and diluted with mobile phase to yield a final concentration of 0.01 mg/ml. Methyl salicylate was added to the solution as an internal standard. The concentration of residual compound was determined by HPLC. On the other hand, degradation compounds were detected by HPLC with direct injection of 10 μl portions of the reaction samples.

2.5.2. Effects of metal ions on degradation

Compound **1** and metal ion concentrations were set at 0.05 mM in pH 7.4 phosphate buffer solution because of the poor solubility of metal ions. The metal ions used were copper(I), copper(II), iron(II) and iron(III) in the forms of CuCl, $CuSO_4$, $FeCl_2$ and $FeCl_3$, respectively. All sample solutions were sealed in clear glass ampules. Degradation was carried out in a constant temperature oil bath at 60° C with shielding from light. The reaction samples were withdrawn at suitable time intervals, cooled on an ice bath, and the concentrations of residual compound and degradation compounds were determined by HPLC with direct injection of 10 μl portions of the reaction samples.

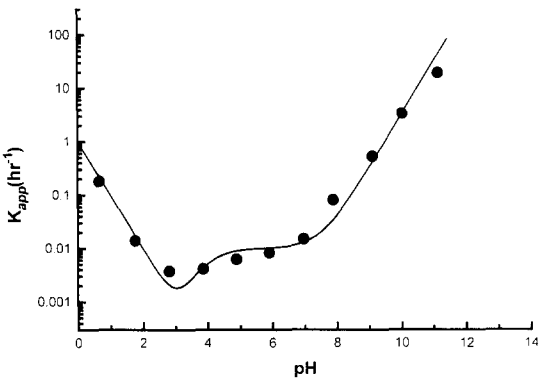

Fig. 1. Log K_{app}-pH profile for degradation of **1** at 60° C in 100 mM buffer solution (ionic strength 0.5). Points are experimental values, and the line is the theoretical curve generated by Eq. 1.

2.6. Degradation of 2,3-dihydro-5-(4-methylphenyl)thiophene-3-carboxylic acid

In this experiment, 0.05 mM 2,3-dihydro-5-(4-methylphenyl)thiophene-3-carboxylic acid (KE-629; **3**) was incubated in pH 7.4 phosphate buffer solution with 0.05 mM copper(II) and iron(III) ion at 60° C with shielding from light. The degradation compounds were analyzed by HPLC.

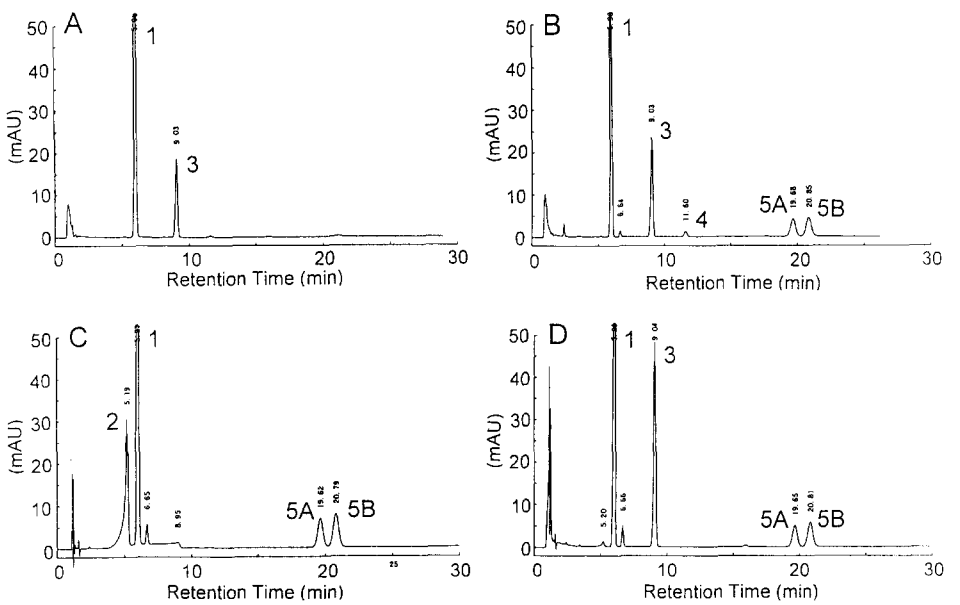

Fig. 2. Typical HPLC chromatograms for the degradation reaction of **1**. HPLC conditions are described in the text. Degradation reaction in: (A) pH 1 solution at 60° C for 48 h, (B) pH 7 at 60° C for 48 h, (C) pH 9 solution at 60° C for 90 min and (D) pH 9 solution with treatment with hydrochloride. (1) **1**; (2) **2**; (3) **3**; (4) **4**; (5A,5B) **5**.

3. Results and discussion

3.1. pH-rate profile

Compound **1** followed pseudo-first-order degradation kinetics in all pH regions at constant temperature. The pseudo-first-order rate constants (K_{app}) were determined from the slopes of semi-logarithmic plots by least-squares regression analysis. The log K_{app}-pH profile, as shown in Fig. 1, suggested that degradation occurred via four routes: (1) specific acid catalysis of unionized species (K_H); (2) specific acid catalysis of ionized species (K'_H); (3) water catalysis of ionized species (K'_{H_2O}); and (4) specific base catalysis of ionized species (K'_{OH}). A similar degradation profile was obtained for aspirin by Edwards (1950). The profile was then fitted to the following equation:

$$K_{app} = K_H \times a_H^2/(a_H + K_a) + (K'_H \times a_H + K'_{H_2O} + K'_{OH} \times a_{OH}) \times K_a/(a_H + K_a) \quad (1)$$

where K_a, a_H, and a_{OH} are the dissociation constant of **1**, hydrogen ion activity and hydroxyl ion activity, respectively. In Fig. 1, the line shown is the theoretical curve obtained using a non-linear least-squares method program developed by Yamaoka et al. (1981), while the points represent experimental results. The reasonable degree of agreement between theoretical and experimental values indicates that Eq. 1 adequately describes the kinetics of degradation.

3.2. Degradation products in aqueous solution

The four major degradation products of **1** in aqueous solution appeared on HPLC (Fig. 2). In the acidic pH region (pH 1–2), 2,3-dihydro-5-(4-methylphenyl)thiophene-3-carboxylic acid (KE-629; **3**) was the degradation product. In the neutral pH region (pH 3–7), the degradation products were **3**, 5-(4-methylphenyl)thiophene-3-carboxylic acid (KE-630; **4**) and 2,2′-dithiomethyl bis[3-(4-methylbenzoyl)propionic acid] (KE-632; **5**), which appeared as two peaks on HPLC depending on which diastereomer was formed. In the alkaline pH region (pH 9–10), a leading peak just before the peak of **1** appeared on HPLC. This product was identified as compound **2**, which is very unstable in acidic solution and rapidly changed to **3**. In addition, in highly alkaline solution (pH > 11), the degradation product was **3**, and **5** did not appear. Table 1 lists the results of assay of **1**, **3–5** and their total concentrations. The total concentrations of **1** and degradation products were above 90% in acidic and neutral pH solutions but less than 90% in alkaline pH solution. These findings indicated that the degradation products of **1** were **3–5** in acidic and neutral pH solutions, whereas unknown degradation products in addition to **2** and **5** were formed in alkaline pH solution. In fact, an unknown peak with a retention time of 6.6 min is present on the HPLC chromatogram of Fig. 2C and D. However, the small quantity of this compound precluded its identification.

Table 1
Assay of compound **1** and its degradation products

Medium	Assay of each compound (%)					
	Time	**1**	**3**	**4**	**5**	Total
Acid (pH 2.2)	0 h	98.2				98.2
(0.1 N HCl)	6 h	94.8	3.6			98.4
	24 h	85.7	12.1			97.8
	48 h	72.5	20.3			92.8
Neutral (pH 7.1)	0 h	99.7				99.7
(pH 7 buffer)	6 h	92.8	4.6			97.4
	24 h	71.4	21.0	0.5	3.2	96.1
	48 h	49.2	25.4	1.7	14.9	91.2
Alkaline (pH 9.2) [a]	0 h	94.7				94.7
(0.1 N NaOH)	30 min	76.2	13.4		2.7	92.3
	60 min	58.3	23.6		5.3	87.2
	90 min	45.1	31.2		8.2	84.5

[a] The concentration in alkaline solution was measured after treatment with hydrochloride.

The degradation products were identified by a comparison of retention times on HPLC chromatograms, HPLC UV spectra and MS spectra obtained by LC/APCI-MS with those of authentic compounds. Good agreement was obtained between the spectrum of each degradation compound and its corresponding authentic compound.

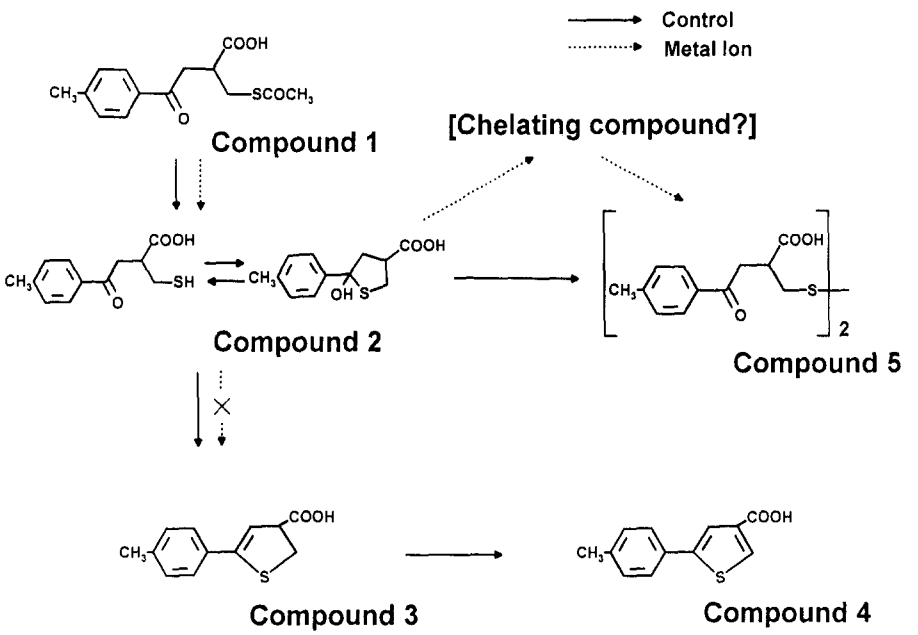

Fig. 3. Degradation pathway of **1** in aqueous solution and **1** in pH 7.4 phosphate buffer solution with the addition of copper(II) ion.

3.3. Degradation pathway for compound 1 in aqueous solution

On the basis of the log K_{app}-pH profile and degradation products identified in solution, the degradation pathway was outlined. Compound **1** is first degraded to **2** by hydrolysis of thio-ester in solutions of all pH values. Compound **2** is changed to **3** by specific acid and base catalysis in acid and alkaline solutions, and **3** is degraded to **4** via oxidation. In neutral pH solution, **2** forms **5** by oxidation. These degradation pathways are demonstrated in Fig. 3.

3.4. Chelating ability of compound 2

Since compound **2** possesses an SH functional group, its chelating ability was studied. If **2** has chelating ability, the degradation products or degradation profile of **1** in aqueous solution should be altered by the addition of metal ions to the reaction solution. The study was performed at physiological pH (7.4). In addition, the reaction temperature was set at 60° C to promote the degradation reactions. The degradation profiles of **1** with and without copper(II) ion in aqueous solution are shown in Fig. 4. These findings show that copper(II) ion did not affect the degradation rate constant of **1**, but did suppress the formation of **3** and promote the formation of **5**. These findings indicated that copper(II) ion does not influence the degradation of **1**, but does affect that of **2**. However, these findings do not clarify

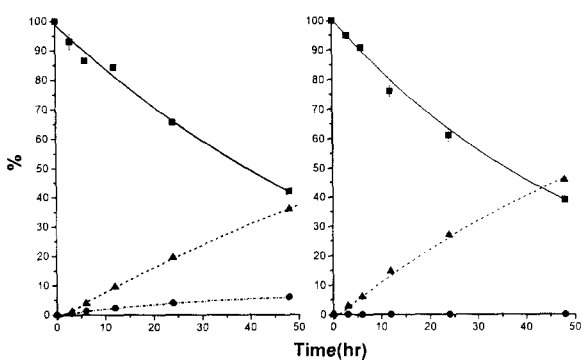

Fig. 4. Degradation profile of **1** at 60° C in pH 7.4 phosphate buffer solution. (Left) Normal degradation pattern; (right) degradation pattern with 0.05 mM copper(II) ion. Each value is the mean ± S.D. of results of triplicate experiments. (■) **1**; (●) **3**; (▲) **5**.

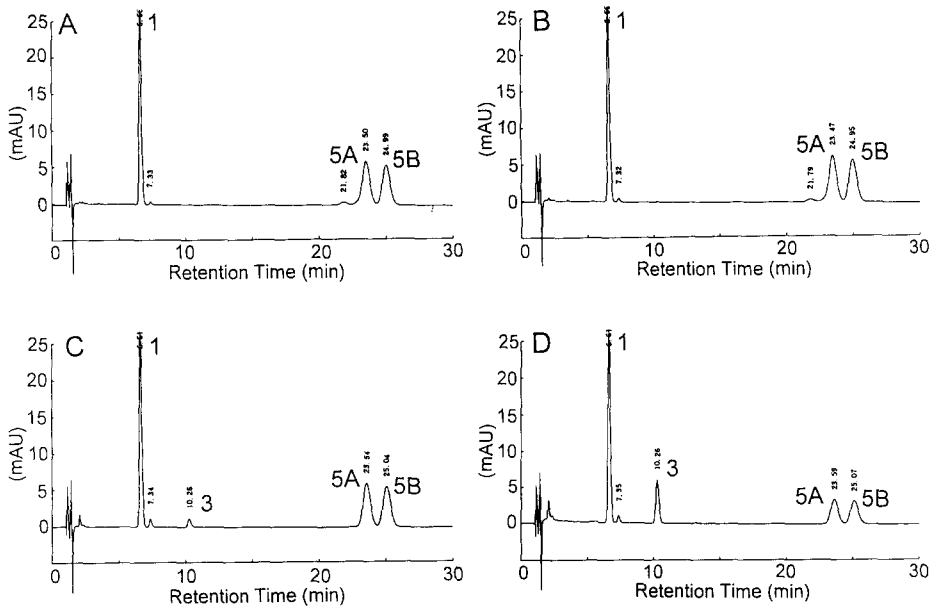

Fig. 5. Typical chromatograms for the degradation reaction of **1** in the presence of several metal ions at 60° C for 48 h. HPLC conditions are described in the text. Degradation reactions with addition of (A) copper(I) ion, (B) copper(II) ion, (C) iron(II) ion, and (D) iron(III) ion. (1) Compound **1**; (2) **2**; (3) **3**; (5A,5B) **5**.

whether **2** has chelating ability, since the formation of **5** may be due to oxidation of metal ion or **3** may be specifically degraded to **5** by metal ion. Therefore, studies of the degradation of **3** with certain metal ions were performed first. The results of those studies showed that **3** was not specifically degraded to **5** by the metal ions. Then studies of stability with other metal ions, copper(I), iron(II) and iron(III), were carried out in aqueous solution. If the formation of **5** was due to the oxidation of metal ions, copper(I) and iron(II) would not have affected the degradation profile of **1**, since they are incapable of oxidation. Some of the chromatograms obtained in this study are shown in Fig. 5. Iron ions did not suppress the formation of **3** in reaction solution, but copper ions did. These findings suggested that suppression of the formation of **3** is not due to oxidation of metal ion. Thus, copper ions may have chelating ability for **2**, and iron(II) may as well, since the formation of **3** was suppressed to a greater extent by iron(II) ion than by iron(III) ion. In each study, little variation was observed in the effect of the several metal ions on the rate constants of degradation of **1**. These findings suggested that **2** possesses chelating ability in the presence of certain metal ions. The degradation pathway of **1** with copper(II) ion is depicted in Fig. 3.

However, chelating compounds did not appear on HPLC chromatograms in this study, and they therefore could not be identified, nor could the stability constant be calculated.

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