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# Physicochemical properties of 2′-benzoyloxycinnamaldehyde

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**Abstract**

2′-Benzoyloxycinnamaldehyde (BCA), a derivative of 2-hydroxycinnamaldehyde demonstrated a potent antitumor effect against several human solid tumor cell lines. The physicochemical properties and degradation kinetics of BCA were investigated to support the drug-development effort. The aqueous solubility of BCA was low, and it was not considered to be hygroscopic. The degradation of BCA followed the first-order kinetics, and the pH-rate profile revealed that the degradation of BCA was governed by general acid- and specific base-catalysis as well as spontaneous hydrolysis. BCA was very unstable in basic conditions, in particular pH above 9, and found to be more stable in acidic conditions such as pH between 2 and 4. The degradation of BCA was accelerated in elevated temperature and high-ionic strength. Therefore, it was suggested that BCA should be stored in slightly acidic conditions with lowered temperature and ionic strength.


*Keywords:* Cinnamaldehyde; Stability; pH-rate profile

## 1. Introduction

*Cinnamomum cassia* Blume (Lauraceae) has been used as a spice in East Asian countries, and also considered to have pharmacological properties such as carminative and antispasmodic (Lee, 2002), thus it has been often combined with other traditional oriental herb medicines. Recently, more pharmacological effects of *C. cassia* have been detailed such as a potent antibacterial activity for *Clostridium perfringens*, *Bacteriodes fragilis* and *Bifidobacterium bifidum* (Lee and Ahn, 1998; Kwon et al., 2003) and a fungicidal activity for *Geotrichum cnadidum*, *Floeckera apivulata*, *Candida lipolytica* and *C. albicans* (Conner and Beuchat, 1984). The extract of *C. cassia* exhibited cytotoxicity against human tumor cells (Kwon et al., 1998; Ka et al., 2003) and antimutagenetic activity (Ohta, 1993), and potentiated the cell-inactivating effect of *cis*-diamminedichloroplatinum (II) in human NHIK 3025 cells (Dornish et al., 1988, 1989). In addi-

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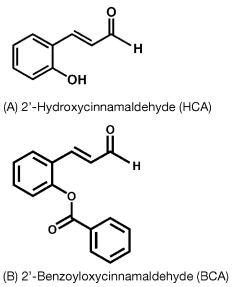


Fig. 1. Structures of: (A) 2′-hydroxycinnamaldehyde (HCA); (B) 2′-benzoyloxycinnamaldehyde (BCA).

tion, it showed an inhibitory effect on farnesyl protein transferase activity in vitro, which relates to immune cell activation as well as carcinogenesis (Koh et al., 1998).

These antitumor effects have been attributed to cinnamaldehydes (CA) such as 2′-hydroxycinnamaldehyde and *o*-methoxycinnamaldehyde (Choi et al., 2001), the major constituents of volatile oil of *C. cassia*. Based on 2′-hydroxycinnamaldehyde (HCA, Fig. 1(A)), CA and related compounds were synthesized from various cinnamic acids, and synthetic CA analogues were studied to develop new anticancer drugs (Kwon et al., 1998). Among them, 2′-benzoyloxycinnamaldehyde (BCA, Fig. 1(B)) strongly inhibited in vitro growth of 29 kinds of human cancer cells, and in vivo growth of SW-620 human tumor xenograft in nude mice (Lee et al., 1999). BCA also showed antiproliferative effect in a proliferative vitreoretinopathy model in the rabbit (Lee et al., 2002).

The potent antitumor effect of BCA showed potential as a new anticancer drug. Thus, in this study the stability of BCA was investigated in different pH buffer systems and at elevated temperature conditions. In addition, basic physicochemical properties of BCA were measured.

## 2. Materials and methods

### 2.1. Materials

BCA was kindly donated from Korea Research Institute of Bioscience and Biotechnology (Taejun, South Korea). Methanol (HPLC-grade) was purchased from Merck (Darmstadt, Germany), and solvents for HPLC were filtered using a 0.45 μm filter and thoroughly degassed in an ultrasonic bath before use. Chromium trioxide, cupric chloride, lithium sulfate, potassium acetate, sodium chloride and boric acid were obtained from Junsei Chemical Co. (Tokyo, Japan), and used as received. All other chemicals were reagent grade and used without further purification.

### 2.2. Methods

#### 2.2.1. Physicochemical property test

The melting point of BCA was measured with a Mel-Temp melting point apparatus (Laboratory Devices Inc., Holliston, MA, USA), fitted with a 0–180 °C mercury thermometer. The solubility of BCA was measured in methanol, ether and distilled water at 25 °C by adding an excess of BCA to each solvent in a 100 ml Erlenmeyer flask. The solutions were mechanically shaken in a water bath (Viscotherm VT 100, Physica Messtechnik GmbH, Ostfildern, Germany) until equilibrium achieved. Samples were filtered through 0.45 μm syringe filter, and the diluted filtrates were subject to analysis. The weight change of BCA in the period of 5-week storage was measured under various relative humidity desiccators: potassium acetate for 21.6%, chromium trioxide for 40%, sodium dichromate for 59.4%, cupric chloride for 68%, sodium chloride for 75.3%, and lithium sulfate for 87.8%.

#### 2.2.2. Stability test

In this study, 0.1 M hydrogen chloride (pH 1), 0.1 M hydrogen chloride–0.1 M potassium chloride (pH 2), 0.1 M hydrogen chloride–0.1 M glycine (pH 3), 0.1 M sodium acetate–0.1 M acetic acid (pHs 4 and 5), 0.1 M potassium phosphate monobasic–0.1 M sodium hydroxide (pH 6–8), and 0.1 M boric acid–0.1 M potassium chloride (pH 9) buffers were used. The ionic strength of the buffer solutions was adjusted to 0.1 with addition of sodium chloride. BCA methanol stock solution (1 mg/mL) was diluted 1000-fold with different pH buffer solutions. A 3 mL aliquot of the diluted buffer solutions was added to 5 mL amber glass ampoules, which were flame sealed and maintained in a constant temperature bath (60 °C) and a light-protected environment.

The temperature dependency of BCA degradation was tested at 40, 60 and 80 °C. BCA methanol stock solution (1 mg/mL) was diluted with distilled water,

and a 3 mL of aliquot was added to 5 mL of amber glass ampoules, which were flame sealed.

The effect of ionic strength on the aqueous stability of BCA at 60 °C was investigated in pH 4.0 in acetate buffer solutions. The effect of ionic strength on reaction rate was ascertained by varying the ionic strength ($\mu$ = 0.1–1.6) with addition of sodium chloride (Chun and Cho, 1995).

*2.2.3. Sample analysis*

The concentration of BCA was determined using a HPLC system, consisted of an LC module I pump, a Waters 486 Tunable UV/visible Absorbance detector, a Waters 746 B data module, an on-line degasser and an auto sampler (Waters, Milford, MA, USA). The compound analysis was carried out using a μ-Bondapak $C_{18}$ column (3.9 × 300 mm i.d., particle size 10 μm, Waters, Milford, MA, USA). The mobile phase consisted of methanol and water (70:30, v/v). Flow rate was 1.0 mL/min and the injection volume was 100 μL, and BCA was detected at 254 nm. The chromatographic peak of BCA was well resolved from interference with a retention time of 7.4 min.

## 3. Results and discussion

### *3.1. Physicochemical property test*

The measured melting point of BCA was 82 ± 0.5 °C. With linearity in the concentration range of 0.1–50 μg/mL, the solubility of BCA was measured 0.4, 18, and 49 mg/mL in water, methanol, and ether, respectively. Aqueous solubility is an important physicochemical parameter affecting pharmacokinetics of a compound, in particular absorption when administered orally. The measured aqueous solubility of BCA was quite low, therefore it requires a pre-formulation effort to achieve a satisfactory aqueous solubility. No significant weight change of BCA was observed under various relative humidity storage conditions (21–88%), hence it was considered to be not hygroscopic.

### *3.2. Stability test*

Fig. 2 shows the % BCA remaining in pH 1–9 at 60 °C. BCA degradation followed the first-order kinetics, and the degradation rate constants were calculated

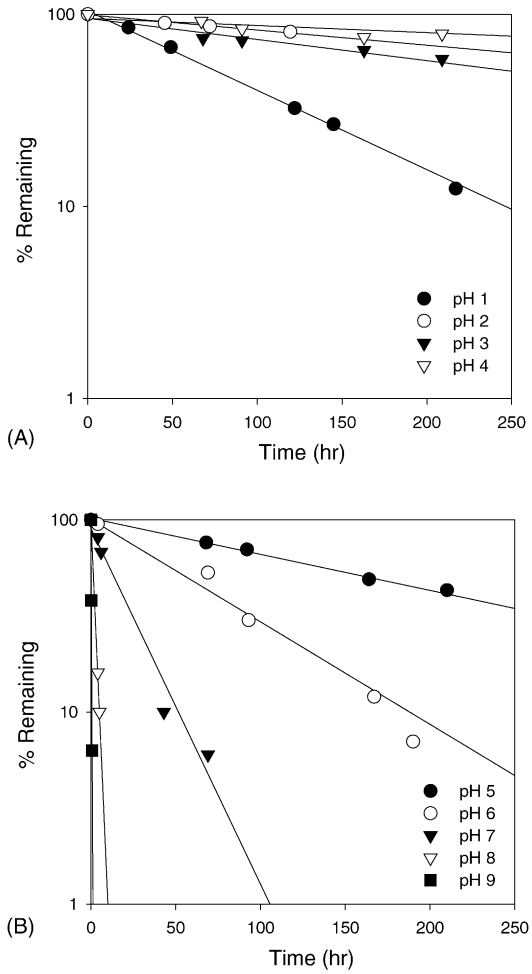


Fig. 2. Percent BCA remaining at 60 °C: (A) in acidic conditions (pH 1–4); (B) in basic conditions (pH 5–9).

from the slopes. BCA was degraded almost instantaneously in pH 10, yielding yellowish precipitations, hence it was not possible to estimate the degradation rate constant. BCA had the fastest degradation rate in pH 9 ($k = 3.6\ h^{-1}$), while it had the slowest degradation rate in pH 4 ($k = 8.9 \times 10^{-4}\ h^{-1}$). The degradation of BCA was relatively slower in acidic conditions such as pH between 2 and 4 (Fig. 2(A)), and in basic conditions such as pH above 7 the degradation was more extensive (Fig. 2(B)).

It is believed that BCA carries a partial charge on the oxygen of carbonyl group depending on the surrounding environment: in acidic condition, the protonated oxygen carries a partial positive charge, and in basic

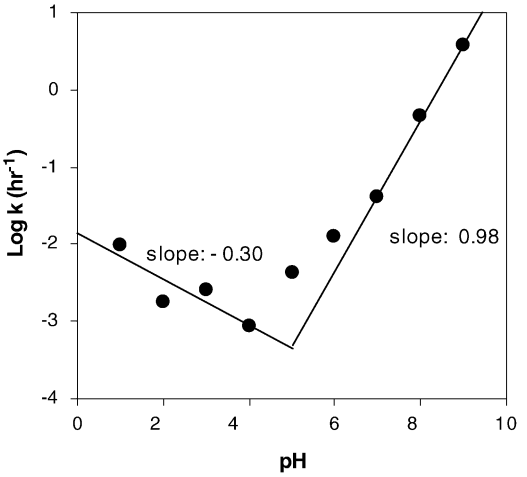


Fig. 3. The effect of pH on BCA degradation: pH-rate profile at 60 °C.

condition, the oxygen is charged partially negatively. In acidic conditions, the attack of water molecule on the carbonyl carbon leads to hydrolysis of the ester bond, while the attack of hydroxide ion leads to the same product in basic conditions. The result of Fig. 2 confirmed that the ester bonds be more vulnerable to hydrolysis in basic conditions.

### 3.3. pH-rate profile

Fig. 3 shows the pH-rate profile BCA degradation at 60 °C. The data points represent first-order rate constants obtained from the initial linear portions of the kinetic plots at each pH. The slope of log *k* against acidic pH (pH < 4) was 0.3, indicating that the degradation reaction followed general acid-catalysis, and one or more buffer components were involved in the degradation of BCA (Kwon et al., 1999). The alkaline portion of the rate-pH profile (pH > 7) showed a slope close to unity (0.98), an indication of specific base-catalysis. The observed deviation of BCA degradation near neutral pH (5 < pH < 7) can be due to extensive spontaneous hydrolysis.

Fig. 4(A) shows % BCA remaining in distilled water at different testing temperatures. Over the temperature ranges of 40–80 °C, the degradation of BCA followed the first-order kinetics. The degradation rate of BCA increased as the temperature increased in distilled water. The degradation rate constant (log *k*) at

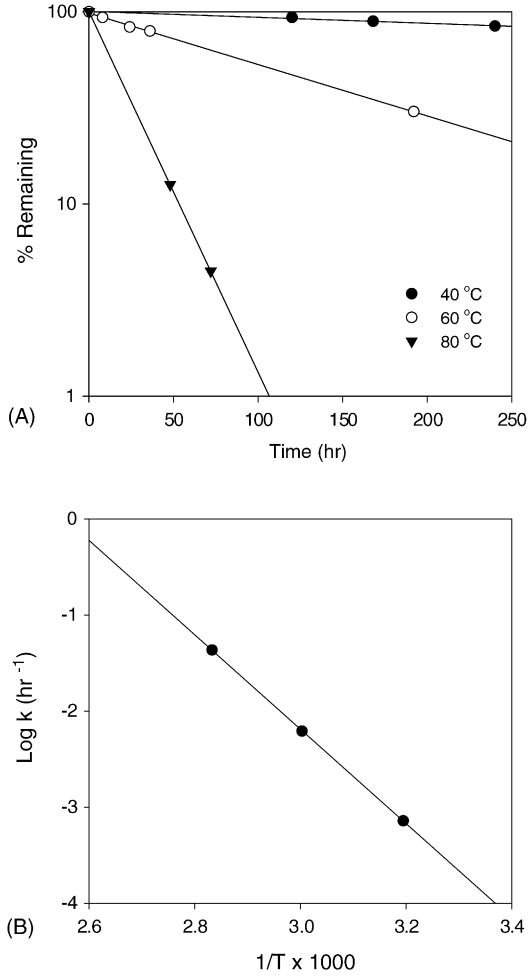


Fig. 4. (A) Percent BCA remaining in distilled water at various temperatures; (B) the effect of temperature on BCA degradation: Arrhenius plot of BCA degradation.

each temperature was plotted against the reciprocal of absolute temperature to estimate $E_a$ (activation energy, kcal/mole) and $A$ (Arrhenius factor, $h^{-1}$) using Arrhenius equation (Fig. 4(B)). The activation energy and Arrhenius factor were determined as 22.17 kcal/mole and $2.55 \times 10^{12}\,h^{-1}$. The extrapolation of the regression curve to 20 °C, the $k_{20}$ °C of BCA in distilled water was estimated as $6.51 \times 10^{-5}\,h^{-1}$. The times to degrade 10% ($t_{10}$, shelf life) and 50% ($t_{50}$, half life) at 20 °C were calculated as 67 and 444 days, respectively.

The effect of ionic strength on the degradation of BCA was investigated in pH 4 buffer solutions at

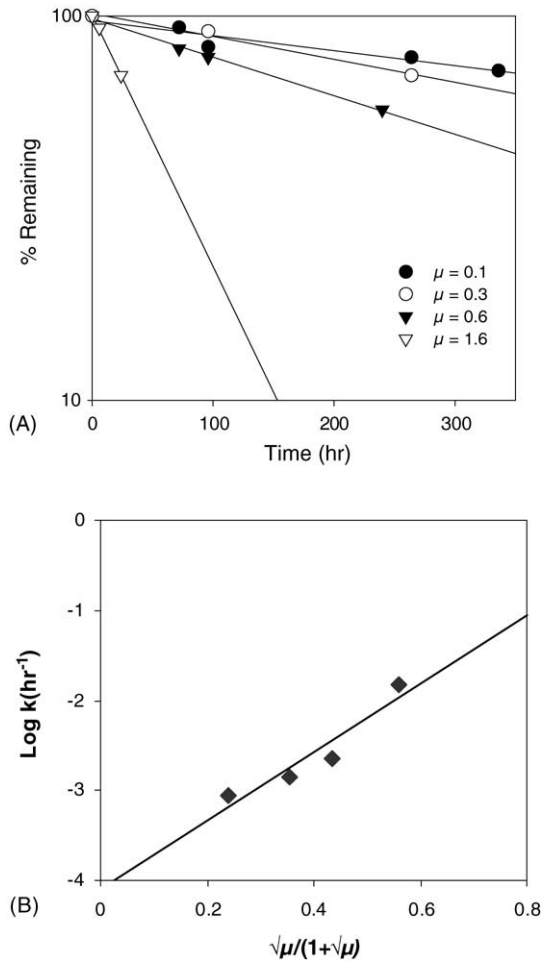


Fig. 5. (A) Percent BCA remaining in pH 4 acetate buffer at 60 °C; (B) the effect of ionic strength on BCA degradation.

60 °C (Fig. 5(A)). The degradation rate increased as the ionic strength increased as shown in Fig. 5(A). Since the ionic strength was greater than 0.1, the modified Debye–Hückel equation was used to establish a relationship between reaction rate constant and ionic strength (Zografi et al., 1964). The plot of log $k$ against $\sqrt{\mu}/(1+\sqrt{\mu})$ showed a positive linear relationship, suggesting that the degradation of BCA was dependent on ionic strength. The dependence of BCA degradation on ionic strength implied that BCA molecule is not neutral under the given condition (pH 4), and verified the partial charge on BCA molecules.

## 4. Conclusions

Basic physicochemical properties and stability of BCA were investigated. The aqueous solubility of BCA was low, and it was not considered to be hygroscopic. The degradation of BCA followed the first-order kinetics, and the pH-rate profile indicated general acid- and specific base-catalysis as well as spontaneous hydrolysis. BCA was very unstable in basic conditions, in particular pH above 9, and found to be more stable in acidic conditions such as pH between 2 and 4. The degradation of BCA was accelerated in elevated temperature and high-ionic strength. Therefore, it was suggested that BCA should be stored in slightly acidic conditions with lowered temperature and ionic strength.

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