International Journal of Pharmaceutics 413 (2011) 63–72

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Improvement of some physicochemical properties of arundic acid, (R)-(−)-2-propyloctanonic acid, by complexation with hydrophilic cyclodextrins

Yuji Miyamoto[a,b], Mai Nakahara[a], Keiichi Motoyama[a], Takako Ishiguro[a,c], Yoshiki Oda[d], Takashi Yamanoi[d], Ichiro Okamoto[e], Akira Yagi[b], Hidekatsu Nishimura[e], Fumitoshi Hirayama[c], Kaneto Uekama[c], Hidetoshi Arima[a,*]

[a] Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan
[b] Pharmaceutical Development Laboratories, Ono Pharmaceutical Co., Ltd., 3-1-1 Sakurai, Shimamoto-cho, Mishima-gun, Osaka 618-8585, Japan
[c] Faculty of Pharmaceutical Sciences, Sojo University, 4-22-1 Ikeda, Kumamoto 860-0082, Japan
[d] The Noguchi Institute, 1-8-1 Koga, Itabashi-ku, Tokyo 173-0003, Japan
[e] Pharmaceutical Engineering Laboratories, Ono Pharmaceutical Co., Ltd., 1-15-26 Kamiji, Higashinari-ku, Osaka 537-0003, Japan

ARTICLE INFO

Article history:
Received 12 January 2011
Received in revised form 9 April 2011
Accepted 13 April 2011
Available online 21 April 2011

Keywords:
Arundic acid
Cyclodextrins
Complexation
Powderization
Solubilization
Taste masking

ABSTRACT

Arundic acid, (R)-(−)-2-propyloctanonic acid, is a novel neurological agent for intractable neurodegenerative diseases. However, arundic acid, an oily drug, has low aqueous solubility and severe bitter/irritating tastes. Consequently, these physicochemical properties of arundic acid need to be improved to develop its pharmaceutical preparations. In the present study, we evaluated whether parent cyclodextrins (CyDs) and 2-hydroxypropylated CyDs (HP-CyDs) can interact with arundic acid, and have powderization, solubilization and taste-masking properties. Of various CyDs, HP-β-CyD had the most potent solubilizing effect for arundic acid. UV and ^{1}H NMR spectroscopic studies demonstrated that arundic acid formed inclusion complexes with CyDs at a molar ratio of 1:1 in solution. The complexation with CyDs changed an oily form of arundic acid to a solid form. The gustatory sensation studies indicate that of various CyDs, HP-β-CyD and γ-CyD showed the most significant taste-masking effects in solution and powders, respectively. HP-β-CyD significantly reduced the response of the electric potential caused by the adsorption of arundic acid to the taste sensor. These results suggest that hydrophilic CyDs have potential as multifunctional excipients for preparing solutions and powders containing arundic acid.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive and intractable disease, which firstly causes muscular depression in the body because of a defect of motor nerves, and gradually causes general muscular depression (Ilieva et al., 2009). For treatment of ALS, liquid medication and/or oral disintegration tablets show great promise in terms of swallowing disability for ALS patients (Nakamura et al., 2002). Arundic acid, (R)-(−)-2-propyloctanonic acid (Fig. 1), is a novel neurological agent for intractable neurodegenerative diseases such as ALS, Alzheimer's disease and Parkinson's disease (Higashino et al., 2009; Himeda et al., 2006; Kato et al., 2004; Mori et al., 2006; Oki et al., 2008). Arundic acid, an oily drug (Fig. 1), is classified as a biopharmaceutics classification system (BCS) II compound, which has high membrane permeability and low aqueous solubility. In addition, arundic acid has a severe bitterness and irritation against tongue, which makes it difficult or unpleasant for patients to take, giving rise to noncompliance. Therefore, it is of great importance to improve the handling and increase aqueous solubility of arundic acid as well as to mask the bitter/irritating tastes at the formulation development phase.

Cyclodextrins (CyDs), cyclic oligosaccharides consisting of usually 6–8 D-glucose units, form inclusion complexes with various molecules not only in aqueous solution but also in the solid state, and are successfully utilized for improvement of pharmaceutical properties of drugs (Stella and Rajewski, 1997; Uekama, 2004; Uekama et al., 1998). Recently, hydrophilic CyD derivatives with high bioadaptability have been developed to accommodate various applications (Loftsson and Duchene, 2007). CyDs are used to improve undesired properties of drugs through the inclusion complexation, such as improvements of solubility, dissolution rate, wettability, and stability. Additionally, CyDs are known to be able to reduce the bitter/irritating tastes of drugs and foods through complexation (Szejtli and Szente, 2005; Ono et al., 2011).

* Corresponding author. Tel.: +81 96 371 4160; fax: +81 96 371 4420.
E-mail address: arimah@gpo.kumamoto-u.ac.jp (H. Arima).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.04.022

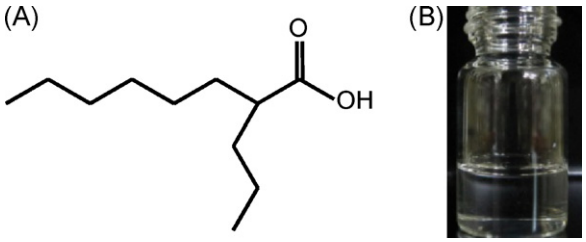

Fig. 1. Chemical structure (A) and appearance (B) of arundic acid.

However, no report has been published as to whether CyDs can improve the undesirable physicochemical properties such as oily form, water-insolubility, and bitter/irritating tastes of arundic acid.

Therefore, in this study, we evaluated the potential use of parent CyDs and 2-hydroxypropylated CyDs (HP-CyDs) to reduce oiliness, and to increase aqueous solubility and to mask bitter/irritating tastes of arundic acid. Firstly, we investigated the complexation of arundic acid with CyDs in aqueous solution and the solid state using the solubility method and the spectroscopic methods. Secondly, we evaluated the effect of CyDs on dissolution rates of arundic acid and its CyD complexes. Finally, we performed the gustatory sensation test in humans and the artificial taste sensor test to examine the masking effects of CyDs on bitter/irritating tastes of arundic acid.

2. Materials and methods

2.1. Materials

Arundic acid was obtained from Ono Pharmaceutical (Osaka, Japan). Parent α-, β-, γ-CyDs, 2-hydroxypropyl-α-CyD (HP-α-CyD, the average degree of substitution (DS) of 2-hydroxypropyl group is 4.0) and 2-hydroxypropyl-β-CyD (HP-β-CyD, the DS value is 4.3) were supplied by Nihon Shokuhin Kako (Tokyo, Japan). Other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

2.2. Solubility methods

The solubility method was conducted according to the method of Higuchi and Connors (1965). An excess amount of arundic acid (10 mg) was added to a test tube containing CyD solutions at various concentrations in water (10 mL), and the mixture was shaken at 25 °C for 7 days. After the equilibrium was attained, the solution was centrifuged at 3000 rpm for 5 min, and an aliquot (0.5 mL) was taken by a cotton-plugged pipette. The methanol containing a 4-hydroxybenzoic acid 2-ethylhexyl as an internal standard (I.S.) was added to the samples to dilute before high-performance liquid chromatography (HPLC) analysis without any crystallization of arundic acid. Then, arundic acid in the sample was analyzed using HPLC under the following conditions: an Intersil GL Science ODS-3V column (5.0 μm, 4.6 mm × 150 mm, Tokyo, Japan), a mobile phase of acetonitrile/20 mM KH_2PO_4 (pH 3.0) (3:2, v/v), a flow rate of 1.0 mL/min, and a detection at a wavelength of 210 nm. The stability constant (K_c) of CyD complexes was calculated by the equation of K_c = slope/[intercept(1 − slope)] using slopes and intercepts of the initial straight-line portion of the phase solubility diagrams.

2.3. Spectroscopic studies

The continuous variation plots (Job, 1928) by the UV method were made under a constant total concentration (10 mM) of host and guest molecules at 210 nm. ^{1}H NMR spectra were taken at 30 °C on a Jeol ECA 600 (Tokyo, Japan), operating at 600 MHz, using a 5-mm sample tube. D_2O (0.6 mL) was used as a solvent, and Na_2CO_3 (0.5 or 1.0 mg) was added in D_2O to dissolve arundic acid. Chemical shifts were expressed in parts per million (ppm) relative to those of the acetone signal (2.225 ppm) with accuracies of ±0.01 ppm. The magnetic field remained stable with the deuterium field lock, confirming negligible change in the signal frequency before and after each experiment. No internal reference was used to avoid interference with binding to CyDs. The concentrations of arundic acid and CyDs were 20 mM/20 mM (arundic acid/α-CyD) and 10 mM/10 mM (arundic acid/HP-β-CyD) in D_2O containing Na_2CO_3 (1.0 mg for arundic acid/α-CyD and 0.5 mg for arundic acid/HP-β-CyD). The NOESY and ROESY spectra were acquired for the arundic acid/α-CyD system and the arundic acid/HP-β-CyD system, respectively, with 64 scans. The relaxation delay was 1.5 s, and the mixing time was set to 2 s. The concentrations of the guest and the host molecules in the NOESY and ROESY spectra were the same as those in ^{1}H NMR spectra.

2.4. Preparation of solid complexes of arundic acid with CyDs

The solid complexes of arundic acid with CyDs were prepared by the kneading method (Tsuruoka et al., 1981) in a molar ratio of 1:1 with a small amount (1 mL) of water. After kneading for 1 h, the resulting slurry was dried under vacuum for 3 days at room temperature. After sieving, the powders with a particle size less than 100 mesh were used for further studies.

2.5. Powder X-ray diffraction study

The powder X-ray diffraction patterns were measured with a Rigaku Rint-2500 diffractometer (Tokyo, Japan) under the following conditions: Ni-filtered Cu Kα radiation (1.542 Å), a voltage of 40 kV, a current of a 40 mA, a divergent slit of 1.74 mm (1°), a scattering slit of 0.94 mm (1°), a receiving slit of 0.15 mm, and a goniometer angular increment of 1°/min.

2.6. Dissolution studies

The dissolution rates of arundic acid and its CyD complexes (equivalent to 10 mg arundic acid) were measured according to the dispersed amount method (Nogami et al., 1969). The drug and its CyD complexes were placed in a jacket beaker and immersed in water (100 mL). The dissolution medium was stirred at 100 rpm at 37 °C. At appropriate intervals, an aliquot (0.5 mL) was withdrawn with a cotton plugged pipette and analyzed for arundic acid by HPLC under the same conditions described above.

2.7. Human gustatory sensation tests

The gustatory sensation tests were performed with five healthy volunteers, who were well trained, according to the previous method (Katsuragi et al., 1997). This investigation was conducted in accordance with the Helsinki Declaration (amendment of Seoul, 2008), and the study protocol was approved by the Ethics Committee of Kumamoto University in Japan (Approval number 273). In the examination, the volunteers were asked to focus on the bitterness and irritation, and not to be influenced by the smell. The concentrations of quinine hydrochloride used as a standard compound were 0.03, 0.1, 0.3 and 1 mM, and the corresponding bitterness and irritation scores were defined as 0, 1, 2, and 3, respectively. Before testing, the volunteers were asked to keep the above standard quinine solutions in their mouths for 10 s, and had to rank each one, according

Table 1
Lipid components for the sensor membranes.

Channel	Sensor	Lipid element	Plasticizer	Charge
1	SB2AC0	Palmitic acid	Dioctyl phenylphosphonate	Negative
2	SB2AN0	Phosphoric acid di-*n*-decyl ester	Dioctyl phenylphosphonate	Negative
5	SB2C00	Tetradodecyl ammonium bromide	2-Nitrophenyl octyl ether	Positive
6	SB2AE1	Tetradodecyl ammonium bromide	Dioctyl phenylphosphonate	Positive

to its bitterness and irritation. Then, the volunteers were asked to rank kneading products (equivalent to 8 mg of arundic acid) or CyDs solutions (5–100 mM) containing 50 mM arundic acid according to their intensity of bitterness and irritation. The volume of solution sample was 10 mL. All samples were kept in the mouth for 10 s. After tasting, the volunteers gargled well before tasting the next sample.

2.8. Artificial taste sensor tests

The taste sensor system, SA402 (Intelligent Sensor Technology, Atsugi, Japan) was used to measure the electric potential of the sample solution (Kataoka et al., 2004; Miyanaga et al., 2003; Takagi et al., 1998; Toko, 1998; Uchida et al., 2001, 2003). The detecting sensors composed of lipids membranes were used in this study (Table 1). The surfaces of the sensor membranes in channels 5 and 6, which can react to bitterness and irritation of drug, were charged positively. The electrode was first dipped into the reference solution (Vr) and then into the sample solution (Vs). The difference between Vs and Vr indicates relative value (Vs − Vr). When the electrode is dipped into the reference solution again, the new potential of the reference solution is defined as Vr′. The difference (Vr − Vr′) between the potentials of the reference solution before and after sample measurements is defined as CPA (change of membrane potential caused by adsorption) value, which corresponds to aftertaste.

2.9. Statistical analysis

Data are given as the mean ± S.E. Statistical significance of means for the studies was determined by analysis of variance followed by Scheffe's test. *p*-Values for significance were set at 0.05.

3. Results and discussion

3.1. Phase solubility diagrams

The solubilizing effects of CyDs on arundic acid in aqueous solution were studied by the solubility method (Higuchi and Connors, 1965). Fig. 2 shows the phase solubility diagrams of arundic acid with parent CyDs, HP-α-CyD and HP-β-CyD in water at 25 °C. The parent CyDs systems showed the B_S-type (Higuchi and Connors, 1965) phase solubility diagrams with arundic acid, while HP-α-CyD and HP-β-CyD provided the A_L-type (Higuchi and Connors, 1965) diagrams. The stability constants (K_c) of the complexes were calculated from the initial straight lines of the diagrams (Table 1). The K_c values of the complexes in the α-CyDs and β-CyDs systems were larger than that of the γ-CyD system, suggesting that α-CyDs and β-CyDs have high ability of complexation with arundic acid in water, compared to γ-CyD. However, the solubilizing effects of parent α-CyD and β-CyD on arundic acid were limited, because of the low solubility of the arundic acid/natural CyDs complexes as estimated from the B_S-type diagrams. Meanwhile, HP-α-CyD and HP-β-CyD

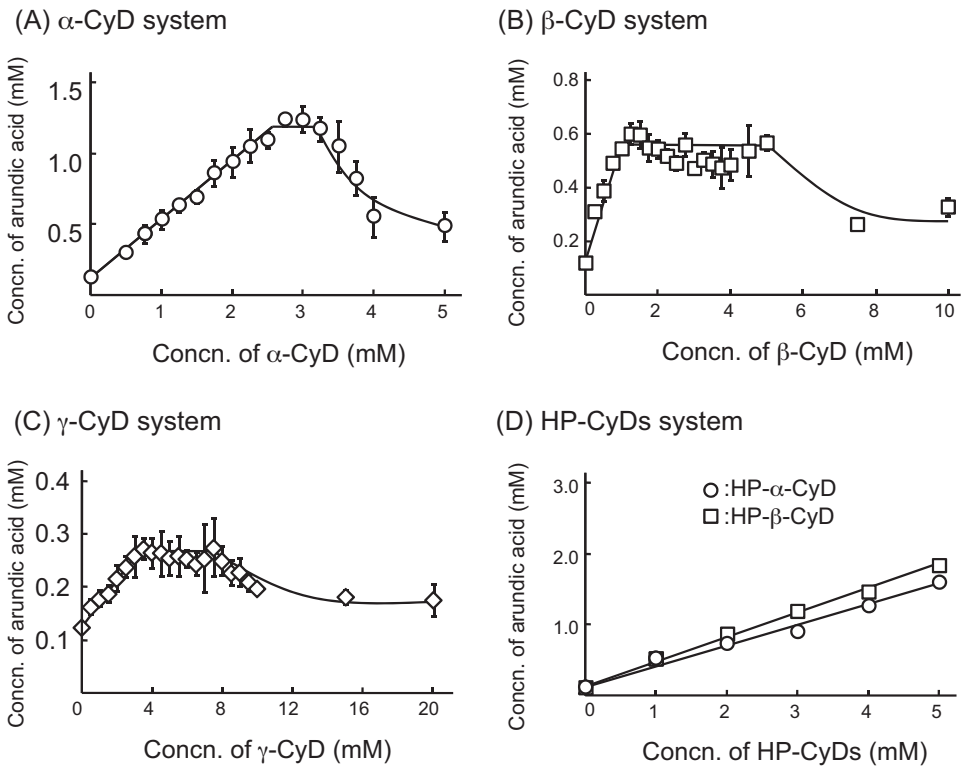

Fig. 2. Phase solubility diagrams of arundic acid/CyDs systems in water. Each point represents the mean ± S.E. of 3-4 experiments. (A) α-CyD system, (B) β-CyD system, (C) γ-CyD system, and (D) HP-CyDs system.

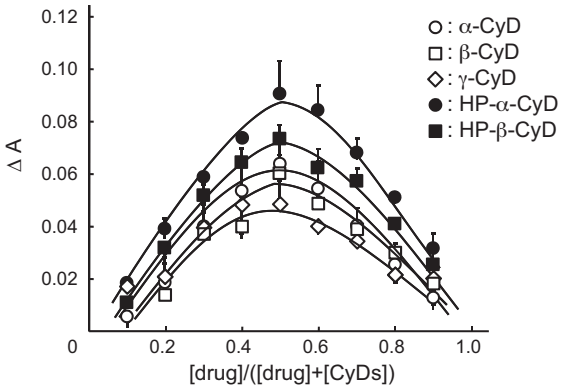

Fig. 3. Continuous variation plots for arundic acid/CyD systems. The total concentrations of arundic acid and CyDs were 10 mM. Each point represents the mean ± S.E. of 3 experiments.

significantly increased the solubility of the arundic acid in water, and the latter tended to show a higher solubilizing effect than that of the former. In fact, enhancement factors (Loftsson and Brewster, 1996), the solubility of the drug in the aqueous CyD solution by its solubility in water, of the complexes of arundic acid with α-CyD, β-CyD, γ-CyD, HP-α-CyD, and HP-β-CyD at 5 mM CyDs were 3.9, 4.6, 2.1, 12.5 and 14.9, respectively. These results suggest that of various CyDs, HP-β-CyD had the most potent solubilizing effect on arundic acid.

3.2. Spectroscopic studies

The phase solubility diagrams (Fig. 2) suggest that the complexes of arundic acid with CyDs are formed at a molar ratio of 1:1, because of the A_L or B_S type of the phase solubility diagrams. To confirm the molar ratio of the complexes in solution, we performed the continuous variation method using the UV spectroscopy in phosphate buffer (pH 7.4). Here, we used a buffer, not water, so that the pH value was not changed by the addition of arundic acid, because the spectra are sensitive to a change in the pH value. From Job's plots (Job, 1928), all of the CyD complexes showed the maximal absorption intensities at the [drug]/([drug] + [CyDs]) value of 0.5 (Fig. 3), suggesting that CyDs form inclusion complexes with arundic acid at a molar ratio of 1:1 in the phosphate buffer.

To gain insight into the inclusion mode of CyDs complexes with arundic acid, one-dimensional and two-dimensional ^{1}H NMR spectroscopic studies were performed. Fig. 4 shows the change in one-dimensional ^{1}H NMR chemical shifts of arundic acid by the addition of α-CyD and HP-β-CyD. Here α-CyD was used in the ^{1}H NMR study, because α-CyD is a pure compound, so it is easy to evaluate the NMR result, and it showed the significant inhibitory effect on bitter/irritating tastes of arundic acid in gustatory sensation test as shown in Figs. 9 and 10, compared with HP-α-CyD, although the ^{1}H NMR study regarding the interaction between arundic acid and HP-β-CyD is required hereafter. Upon the addition of α-CyD, large low-field shifts were observed in the H3-H8 and H1′–H3′ protons of arundic acid, and a high-field shift was shown in H2 proton of arundic acid (Fig. 4A). An almost identical result was observed in the HP-β-CyD system, although a chemical shift of the H2 proton was not observed (Fig. 4B). These chemical shifts may be due to the solvent effect, conformation change and the steric compression effect through inclusion complexation (Uekama et al., 1998). Moreover, CyDs might somewhat dissociate a micelle structure of arundic acid derived from its amphiphilicity. Next, we conducted the two-dimensional NOESY and ROESY spectroscopic studies to estimate inclusion modes of arundic acid in the α-CyD and HP-β-CyD cavities, respectively (Fig. 5). Here, the

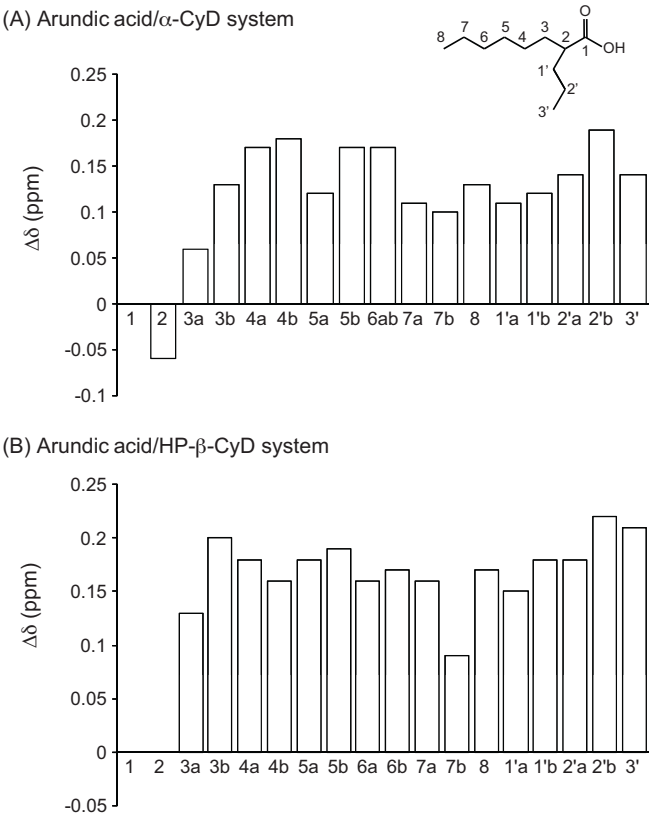

Fig. 4. Effects of CyDs (5 mM) on ^{1}H NMR chemical shifts of arundic acid (5 mM) in D_2O containing 10% DMSO-d_6. (A) The α-CyD system and (B) the HP-β-CyD system. $\Delta\sigma = \sigma_{\text{with CyD}} - \sigma_{\text{drug alone}}$. Each value represents the mean ± S.E. of 3 experiments.

difference in the two-dimensional ^{1}H NMR spectroscopic methods between the α-CyD and HP-β-CyD systems resulted from the accuracy of the resulting data. In the NOESY spectrum, the H2 and H3 protons of α-CyD gave the correlation peaks with the H3–H7 and H3′ protons of arundic acid (Fig. 5A and B), and H5 proton of α-CyD provided the crosspeaks with H8 proton of arundic acid (Fig. 5B). These results suggest that the main aliphatic chain (H2–H8) of the arundic acid molecule is included in the α-CyD cavity, and the H8 and H3′ protons of arundic acid locate around the primary and secondary hydroxyl groups of the α-CyD molecule, respectively. However, H5 proton of α-CyD elicited the crosspeak with H3′ of arundic acid, suggesting that the inclusion mode has the opposite direction of arundic acid, i.e. the H8 and H3′ protons of arundic acid locate around the secondary and primary hydroxyl groups of the α-CyD molecule, respectively. In the ROESY spectrum, the H5 proton of HP-β-CyD gave the crosspeaks with the H8 proton of arundic acid (Fig. 5C), and the H6a and H6b protons of HP-β-CyD showed the crosspeaks with the H8 protons of arundic acid (Fig. 5C). Also, the H3 proton of HP-β-CyD provided the crosspeak with H8 proton of arundic acid (Fig. 5C and D) and with its H3–H8 protons (Fig. 5E). These results suggest that the main aliphatic chain (H3–H8) partially enters the HP-β-CyD cavity, compared with in the α-CyD cavity, reflecting the lack of chemical shift of the H2 proton of arundic acid (Fig. 4B). However, the H5, H6a and H6b protons of HP-β-CyD also gave the crosspeaks with the H3′ protons of arundic acid (Fig. 5C), suggesting that the short chain of arundic acid may be included in the HP-β-CyD cavity. Meanwhile, the continuous variation method using the UV spectroscopy showed the inclusion complex at a molar ratio of 1:1 (Fig. 3). Hence, the inclusion modes of arundic acid in the α-CyD and HP-β-CyD cavities are highly unlikely to be simple, and the detailed inclusion modes such as orientation and location of arundic acid into these CyD cavities

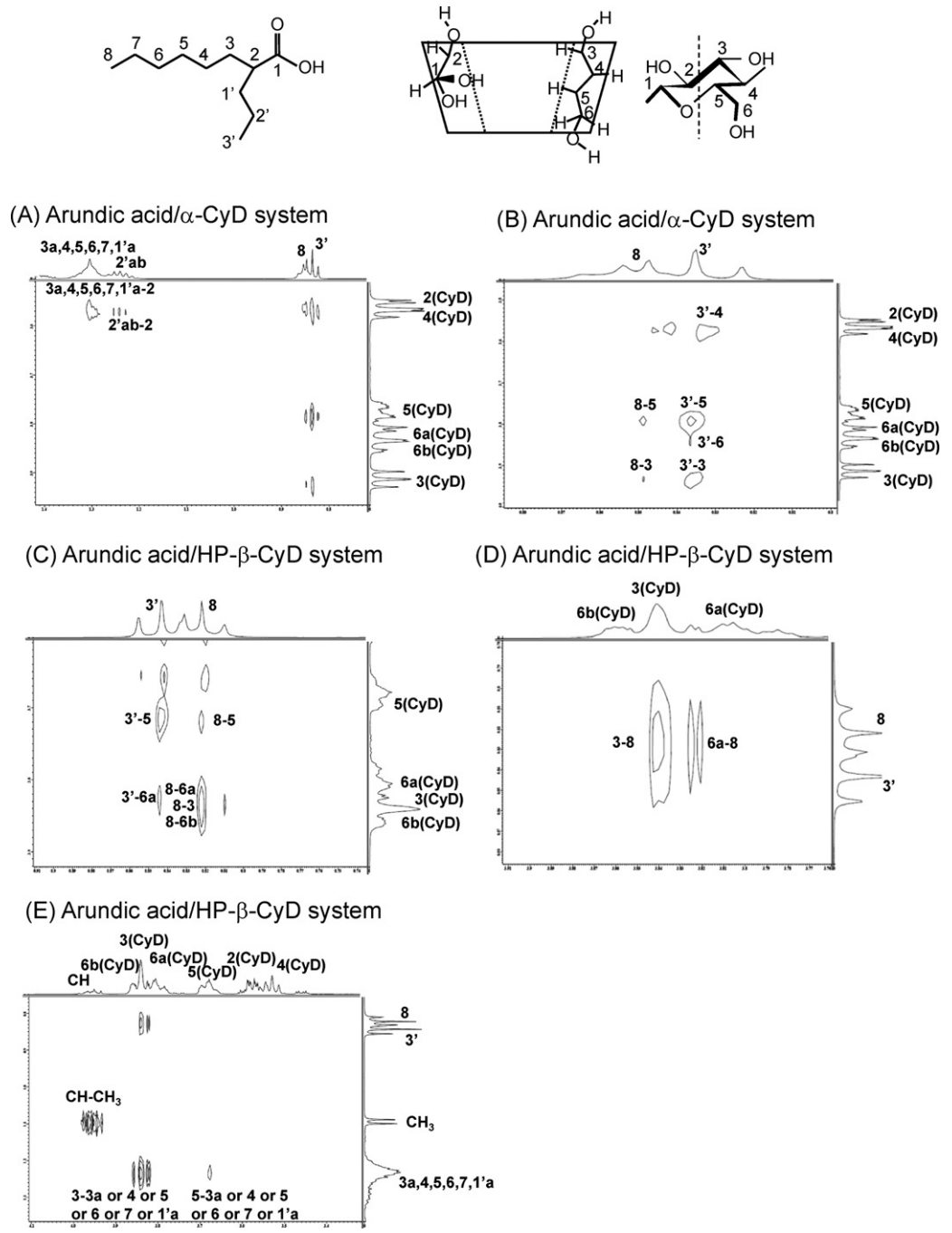

Fig. 5. Partial counter plots of NOESY and ROESY spectra. (A and B) The NOESY spectra of the arundic acid/α-CyD system. (C–E) The ROESY spectra of the arundic acid/HP-β-CyD system. The concentrations of α-CyD and HP-β-CyD were 20 mM and 10 mM, respectively. The molar ratio of arundic acid and CyDs was 1:1.

remain unclear. Therefore, further elaborate studies are required to clarify the precise inclusion modes.

To investigate whether CyDs can interact with arundic acid in the solid state, we prepared kneading products of arundic acid with CyDs at a molar ratio of 1:1. All of the kneading products showed powder forms (Fig. 6), indicating that CyDs improve a handling of arundic acid because of a conversion of arundic acid from an oily form to a powder form. Next, we performed the powder X-ray diffraction studies (Fig. 7). Arundic acid alone could not be measured because of an oily drug. It is well known that the natural CyDs show many crystalline peaks. Likewise, the physical mixtures of arundic acid with natural CyDs showed almost the same patterns as natural CyDs, because the peak derived from arundic acid did not appear and the interaction between them was weak. Meanwhile, kneading products of arundic acid with natural CyDs elicited different powder X-ray diffraction patterns from those of natural CyDs and the physical mixtures (Fig. 7). In sharp contrast, HP-α-CyD and HP-β-CyD alone, physical mixtures and kneading products showed halo patterns, most probably due to amorphous forms (Fig. 7D and E), because the HP-CyDs are mixtures with different DS values. Therefore, it is unclear whether the kneading products of arundic acid with HP-α-CyD and HP-β-CyD are inclusion complexes in the solid state. In addition, thermal analysis would be also useless for a confirmation of the complexation in the present cases, because

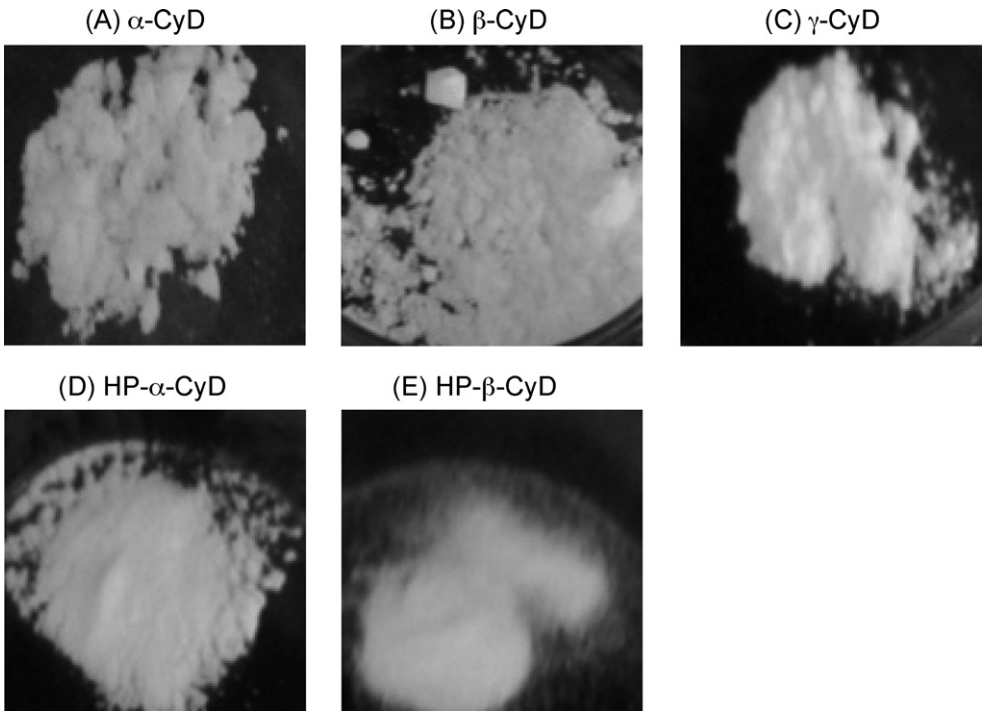

Fig. 6. Appearance of the kneading products of arundic acid with various CyDs. (A) α-CyD, (B) β-CyD, (C) γ-CyD, (D) HP-α-CyD and (E) HP-β-CyD.

of amorphous complexes. Thereafter, to reveal the complexation of arundic acid with HP-α-CyD and HP-β-CyD in detailed should therefore be characterized by solid-state ^{13}C NMR analysis. However, we demonstrated that HP-α-CyD and HP-β-CyD can form complexes in the phase solubility diagrams. Eventually, it is presumed that HP-α-CyD and HP-β-CyD as well as parent CyDs form complexes with arundic acid in the solid state.

3.3. Dissolution tests

To improve the dissolution property of arundic acid, the dissolution rates of arundic acid alone and its CyD complexes were measured in water at 37 °C (Fig. 8). Here we used the disperse amount method (Nogami et al., 1969) for a dissolution study because it is a traditional and common method. In this method,

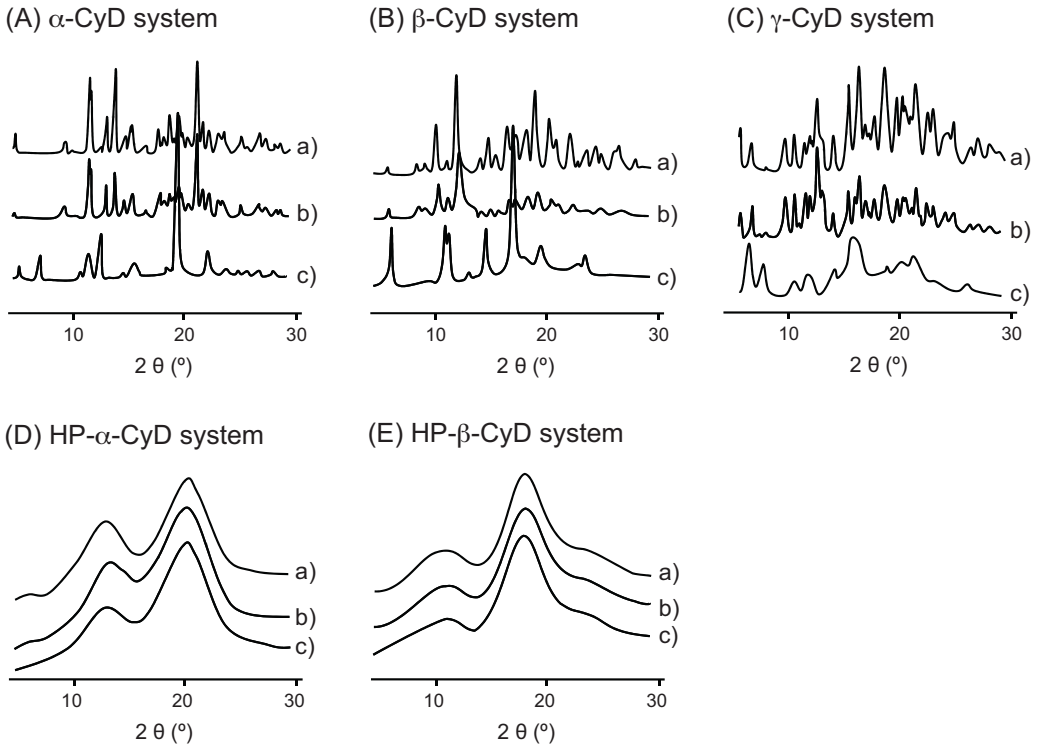

Fig. 7. Powder X-ray diffraction patterns of the arundic acid/CyDs systems. (A) α-CyD system, (B) β-CyD system, (C) γ-CyD system, (D) HP-α-CyD system and (E) HP-β-CyD system. (a) CyD alone, (b) arundic acid/CyD physical mixture, and (c) arundic acid/CyD kneading product.

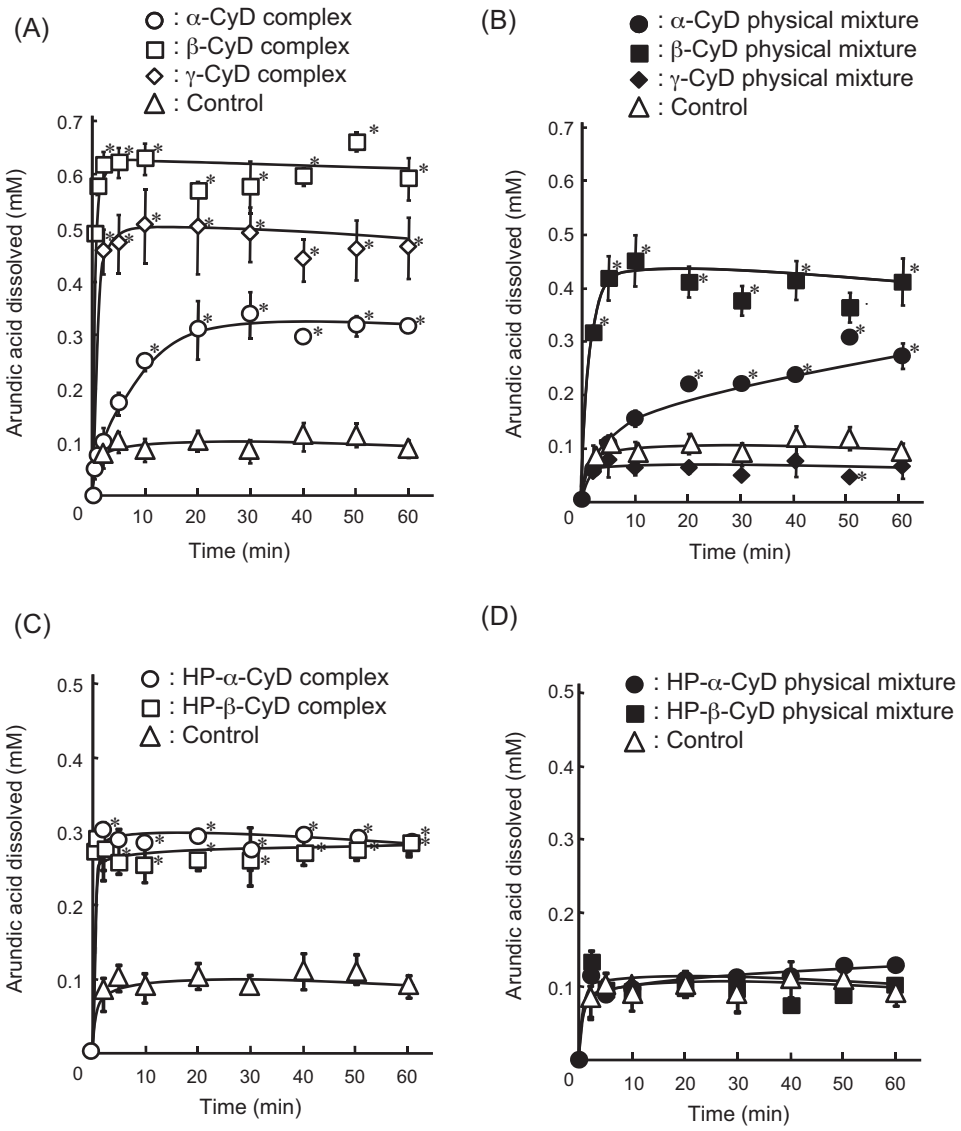

Fig. 8. Dissolution profiles of arundic acid from the physical mixtures and complexes of arundic acid with CyDs. Each point represents the mean ± S.E. of 3–4 experiments. $^{*}p < 0.05$ versus control (arundic acid/lactose physical mixture).

an initial region of the dissolution curve is believed to be the sink condition. Thereby, we evaluated dissolution rates of arundic acid and the CyD complexes using the initial regions. In the natural CyDs complexes, the dissolution rates increased in the order of control (arundic acid alone) < α-CyD complex < γ-CyD complex < β-CyD complex. This order was inconsistent with the magnitude of the stability constants (Table 2). Interestingly, the dissolution rates in the physical mixture systems also increased in the order of γ-CyD physical mixture ≅ control < α-CyD physical mixture < β-CyD physical mixture. The reason why the physical mixtures with α-CyD and β-CyD augmented the dissolution rates of arundic acid is still unknown. One possibility may be that during the preparation of physical mixtures, the complexes were formed. However, this possibility can be discounted, because the powder X-ray diffraction patterns of the physical mixture were totally different from those of the complexes with α-CyD and β-CyD (Fig. 8). Thereafter, further studies are required to clarify the mechanism. Meanwhile, in the HP-CyDs systems, the dissolution rates of the HP-α-CyD complex and HP-β-CyD complex increased markedly, compared to that of control, but the enhancing effects of the two HP-CyDs complexes were almost equivalent, despite the K_c value of the HP-β-CyD complex was higher than that of the HP-α-CyD complex. Contrary to the parent CyDs system, the dissolution rates of the physical mixtures with HP-α-CyD and HP-β-CyD did not further increase the dissolution rate of arundic acid, suggesting that HP-α-CyD and HP-β-CyD complexes have different physicochemical properties from these physical mixtures. Actually, as shown in Fig. 7, the HP-α-CyD complex and HP-β-CyD complex were amorphous, thereby we estimated the rapid dissolution of the complexes. Contrary to our expectations, the dissolution rates of HP-α-CyD complex and HP-β-CyD complex were, however, significantly lower than those of the β-CyD complex and γ-CyD complex. It therefore seems evident that the complexation with these CyDs plays a crucial role in

Table 2
Stability constants for inclusion complexes of arundic acid with CyDs.

CyD	K_c (M^{-1})
α-CyD	1897 ± 51
β-CyD	1967 ± 21
γ-CyD	344 ± 81
HP-α-CyD	1608 ± 95
HP-β-CyD	1815 ± 27

Each value represents the mean ± S.E. of 3–4 experiments.

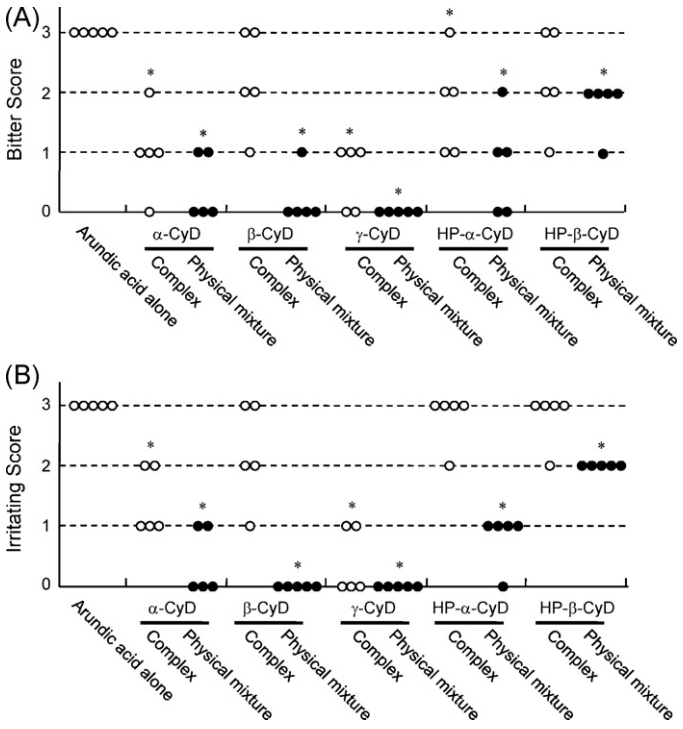

Fig. 9. Effects of CyDs on bitterness score (A) and irritation score (B) of arundic acid in the physical mixtures and kneading products in healthy volunteers. $^{*}p < 0.05$ versus arundic acid alone.

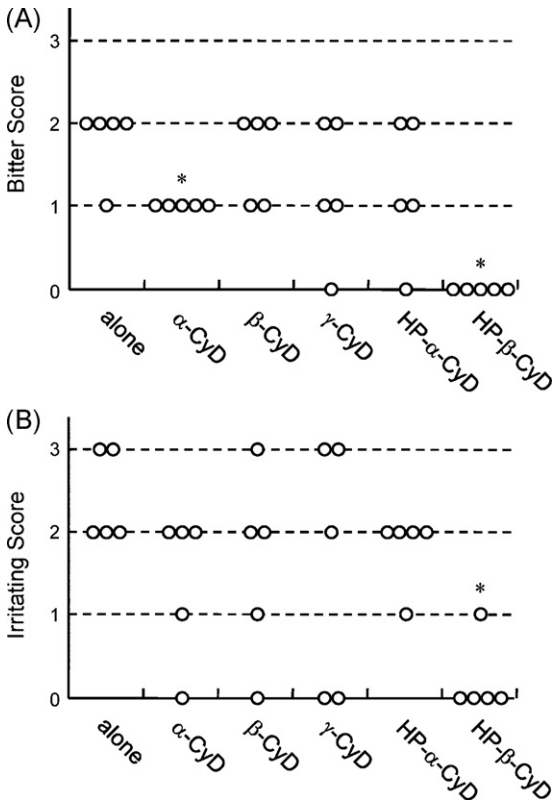

Fig. 10. Effects of CyDs on bitterness score (A) and irritation score (B) of arundic acid in solutions in healthy volunteers. $^{*}p < 0.05$ versus arundic acid alone.

increasing the dissolution rates of arundic acid, although the effects of CyDs on the dissolution properties of arundic acid were very complicated. Probably, various physicochemical properties such as wettability, particle sizes, viscosity, microscopic structures, viscosities of the complexes and physical mixtures may be involved in the intricate effects of CyDs on the dissolution rate and stability constants. Studies are needed to decipher the mechanisms. Furthermore, it is important to compare the dissolution properties between the complexes prepared by a kneading method and those prepared by other methods such as solvent evaporation or freeze drying method, and so we are planning to do the pivotal study in the next study. Anyhow, these results suggest that the complexation with CyDs enhances the dissolution rate of arundic acid.

3.4. Gustatory sensation study

To investigate whether CyDs can suppress bitter/irritating tastes of arundic acid in the mouth, the gustatory sensation study was conducted by five healthy volunteers. Fig. 9A and B shows relative bitterness and irritating scores for the complexes and the physical mixtures of arundic acid with CyDs in the solid state, respectively. Reflecting severe bitter/irritation tastes, arundic acid alone provided the maximal score 3 for both bitterness and irritation (Fig. 9). As a whole, the complexes and physical mixtures reduced the bitterness and irritation scores, compared with arundic acid alone, and the suppressive effects of CyDs on both scores were consistent. It is of note that the parent CyDs systems reduced the bitter/irritating tastes more than the HP-CyDs systems. Interestingly, of the parent CyD systems, the physical mixtures elicited bitter/irritating scores less than the CyD complexes, especially the physical mixture with γ-CyD eliminated the bitter/irritating tastes of arundic acid. These suppressive effects of CyDs on bitter/irritating tastes of arundic acid may be ascribed to the inhibition of the binding of arundic acid to taste receptors on tongue, because of (1) the slow release of the arundic acid from the physical mixtures, compared to that from the complexes, (2) the masking effects of CyDs on binding site of arundic acid to the taste receptors and (3) sweetness of CyDs themselves (Szejtli and Szente, 2005).

Next, to reveal the suppressive effects of CyDs on the bitter/irritating tastes of arundic acid in liquid medication and to gain insight into the mechanism by which CyDs suppress the bitter/irritating tastes of arundic acid in powder form, we evaluated the effects of CyDs on bitter/irritating tastes of arundic acid in solutions (Fig. 10). In sharp contrast to the results of powder forms of CyDs used in this study, HP-β-CyD reduced the bitter/irritating tastes of the arundic acid most potently, and the suppressive effect of HP-β-CyD was in a concentration-dependent manner (data not shown). The strongest suppressive effect of HP-β-CyD was consistent with the magnitude of the K_c value (Table 2), so the effect could be ascribed to the inclusion complexation of arundic acid with HP-β-CyD. Meanwhile, α-CyD showed the second-highest suppressive effect, following HP-β-CyD, even though α-CyD had higher stability constant with arundic acid (Table 2). This slight inhibitory effect of α-CyD might be attributed to the difference in the amount of free drug in an oral cavity: the α-CyD system may provide free drugs at a high extent after dissolution in an oral cavity, compared to the HP-β-CyD system, possibly due to the competitive inclusion between α-CyD and the other physiological components in saliva. In addition, it is possible that HP-β-CyD may affect function of T2R receptor, which is a G protein-coupled receptor (GPCR) localizing in lipid rafts on plasma cell membranes of taste cells, because β-CyDs are known to suppress GPCR function in various cells (Patel et al., 2008) as described below. The differences in the magnitude of the suppressive effects of CyDs between the powder form and solution could be attributed to the absence and presence of the dissolution process. Namely, the slower the dissolution rates of CyD complexes and the higher the stability constants of CyD complexes with arundic acid in solution state, the weaker the

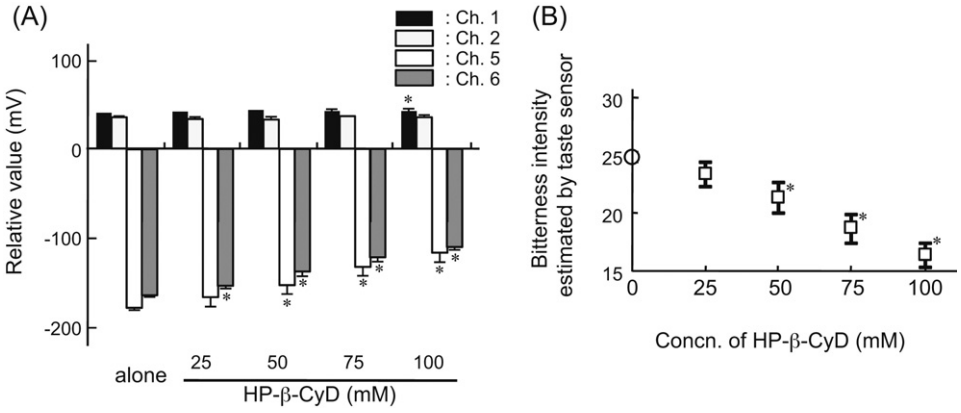

Fig. 11. Effects of HP-β-CyD on relative value of taste sensor (A) and estimated bitterness intensity (B), caused by arundic acid. Each value represents the mean ± S.E. of 3–7 experiments. *$p < 0.05$ versus arundic acid alone.

interaction of arundic acid with taste buds and the bitter/irritating tastes.

However, these above results clearly demonstrate that the suppressive mechanisms of CyDs on the bitter/irritating tastes of arundic acid are not simple. Recently, bitter transduction in mammalian taste receptor cells has been mediated by G protein and GPCRs (Lindemann, 1996; Wong et al., 1996). In addition, T2Rs in humans and mice are genetically linked to loci associated with bitter perception (Capeless et al., 1992; Conneally et al., 1976; Reed et al., 1999), and are selectively expressed in taste receptor cells implicated in bitter transduction (Adler et al., 2000; Chandrashekar et al., 2000; Ming et al., 1998; Wong et al., 1996; Zhang et al., 2003). Meanwhile, the widespread use of β-CyDs for change in the structure and the function of lipid rafts, where concentrate glycosylphosphatidylinositol-anchored protein and/or GPCRs, has been well known (Simons and Ehehalt, 2002; Simons and Toomre, 2000). These lines of evidence make it tempting to speculate that the reduction of the bitter/irritating tastes of arundic acid by the addition of CyDs was responsible for (1) prevention of the contact of arundic acid to T2Rs and (2) interaction of HP-β-CyD with lipid rafts localized taste receptors cells. However, the short contact time of CyDs with membranes of taste bud cells may not exert the inhibitory effect of CyDs on bitter/irritating tastes of arundic acid. Anyhow, to address the hypothesis, we are currently studying the effects of HP-β-CyD on bitter transduction via T2R using T2R overexpressing cells. Collectively, the results of the gustatory sensation study suggest that γ-CyD and HP-β-CyD in powder form and in solution, respectively, have the potential for the suppression of bitter/irritating tastes of arundic acid in humans.

3.5. Taste sensor test

An artificial taste sensor is reported to be a useful tool to quantify the intensity of bitterness and sweetness of drugs and foods, especially in solution (Miyanaga et al., 2002; Nakamura et al., 2002; Uchida et al., 2001). Therefore, we evaluated the taste-masking ability of HP-β-CyD for arundic acid using an artificial taste sensor in order to confirm the result of gustatory sensation study in solution (Fig. 11). There is no significant change in the relative values against negative charge channels (Channels 1 and 2) between the systems with and without HP-β-CyD. Meanwhile, HP-β-CyD significantly suppressed the relative values of arundic acid against positive charge channels (Channels 5 and 6) in a concentration-dependent manner (Fig. 11A). We also confirm that HP-β-CyD suppressed the CPA values, which correspond to aftertaste, against arundic acid alone (data not shown). Also, HP-β-CyD significantly decreased the bitterness intensity estimated by taste sensor in a concentration-dependent manner (Fig. 11B). These results suggest that bitter/irritating tastes of arundic acid were reduced by the complex formation with HP-β-CyD in solution, expecting that HP-β-CyD suppresses the bitter/irritating tastes of arundic acid in gustatory sensation studies. From here on, we are planning to reveal the taste-masking effect of CyDs on arundic acid in the powder form using a novel taste sensor and a disintegration testing apparatus (Harada et al., 2010).

4. Conclusions

In the present study, we have demonstrated that hydrophilic CyDs have not only powderizing and solubilizing effects on arundic acid but also a taste-masking effect through the complexation of CyDs with arundic acid. Therefore, these results suggest that hydrophilic CyDs have the potential as multifunctional excipients for preparing solutions and powders containing arundic acid.

References

Adler, E., Hoon, M.A., Mueller, K.L., Chandrashekar, J., Ryba, N.J., Zuker, C.S., 2000. A novel family of mammalian taste receptors. Cell 100, 693–702.
Capeless, C.G., Whitney, G., Azen, E.A., 1992. Chromosome mapping of Soa, a gene influencing gustatory sensitivity to sucrose octaacetate in mice. Behav. Genet. 22, 655–663.
Chandrashekar, J., Mueller, K.L., Hoon, M.A., Adler, E., Feng, L., Guo, W., Zuker, C.S., Ryba, N.J., 2000. T2Rs function as bitter taste receptors. Cell 100, 703–711.
Conneally, P.M., Dumont-Driscoll, M., Huntzinger, R.S., Nance, W.E., Jackson, C.E., 1976. Linkage relations of the loci for Kell and phenylthiocarbamide taste sensitivity. Hum. Hered. 26, 267–271.
Harada, T., Uchida, T., Yoshida, M., Kobayashi, Y., Narazaki, R., Ohwaki, T., 2010. A new method for evaluating the bitterness of medicines in development using a taste sensor and a disintegration testing apparatus. Chem. Pharm. Bull. 58, 1009–1014.
Higashino, H., Niwa, A., Satou, T., Ohta, Y., Hashimoto, S., Tabuchi, M., Ooshima, K., 2009. Immunohistochemical analysis of brain lesions using S100B and glial fibrillary acidic protein antibodies in arundic acid- (ONO-2506) treated stroke-prone spontaneously hypertensive rats. J. Neural Transm. 116, 1209–1219.
Higuchi, T., Connors, K.A., 1965. Phase-solubilty techniques. Adv. Anal. Chem. Instr. 7, 117–212.
Himeda, T., Kadoguchi, N., Kamiyama, Y., Kato, H., Maegawa, H., Araki, T., 2006. Neuroprotective effect of arundic acid, an astrocyte-modulating agent, in mouse brain against MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) neurotoxicity. Neuropharmacology 50, 329–344.
Ilieva, H., Polymenidou, M., Cleveland, D.W., 2009. Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. J. Cell Biol. 187, 761–772.
Job, P., 1928. Formation and stability of inorganic complexes in solutions. Justus Liebigs Ann. Chem. 9, 113–203.
Kataoka, M., Miyanaga, Y., Tsuji, E., Uchida, T., 2004. Evaluation of bottled nutritive drinks using a taste sensor. Int. J. Pharm. 279, 107–114.
Kato, H., Kurosaki, R., Oki, C., Araki, T., 2004. Arundic acid, an astrocyte-modulating agent, protects dopaminergic neurons against MPTP neurotoxicity in mice. Brain Res. 1030, 66–73.
Katsuragi, Y., Mitsui, Y., Umeda, T., Otsuji, K., Yamasawa, S., Kurihara, K., 1997. Basic studies for the practical use of bitterness inhibitors: selective inhibition of bitterness by phospholipids. Pharm. Res. 14, 720–724.

Lindemann, B., 1996. Chemoreception: tasting the sweet and the bitter. Curr. Biol. 6, 1234–1237.
Loftsson, T., Brewster, M.E., 1996. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci. 85, 1017–1025.
Loftsson, T., Duchene, D., 2007. Cyclodextrins and their pharmaceutical applications. Int. J. Pharm. 329, 1–11.
Ming, D., Ruiz-Avila, L., Margolskee, R.F., 1998. Characterization and solubilization of bitter-responsive receptors that couple to gustducin. Proc. Natl. Acad. Sci. U.S.A. 95, 8933–8938.
Miyanaga, Y., Inoue, N., Ohnishi, A., Fujisawa, E., Yamaguchi, M., Uchida, T., 2003. Quantitative prediction of the bitterness suppression of elemental diets by various flavors using a taste sensor. Pharm. Res. 20, 1932–1938.
Miyanaga, Y., Tanigake, A., Nakamura, T., Kobayashi, Y., Ikezaki, H., Taniguchi, A., Matsuyama, K., Uchida, T., 2002. Prediction of the bitterness of single, binary- and multiple-component amino acid solutions using a taste sensor. Int. J. Pharm. 248, 207–218.
Mori, T., Town, T., Tan, J., Yada, N., Horikoshi, Y., Yamamoto, J., Shimoda, T., Kamanaka, Y., Tateishi, N., Asano, T., 2006. Arundic acid ameliorates cerebral amyloidosis and gliosis in Alzheimer transgenic mice. J. Pharmacol. Exp. Ther. 318, 571–578.
Nakamura, T., Tanigake, A., Miyanaga, Y., Ogawa, T., Akiyoshi, T., Matsuyama, K., Uchida, T., 2002. The effect of various substances on the suppression of the bitterness of quinine-human gustatory sensation, binding, and taste sensor studies. Chem. Pharm. Bull. 50, 1589–1593.
Nogami, H., Nagai, T., Yotsuyanagi, Y., 1969. Dissolution phenomena of organic medicinals involving simultaneous phase changes. Chem. Pharm. Bull. 17, 499–509.
Oki, C., Watanabe, Y., Yokoyama, H., Shimoda, T., Kato, H., Araki, T., 2008. Delayed treatment with arundic acid reduces the MPTP-induced neurotoxicity in mice. Cell. Mol. Neurobiol. 28, 417–430.
Ono, N., Miyamamoto, Y., Ishiguro, T., Motoyama, K., Hirayama, F., Iohara, D., Seo, H., Tsuruta, S., Arima, H., Uekama, K., 2011. Reduction of bitterness of antihistaminic drugs by complexation with β-cyclodextrins. J. Pharm. Sci. 100, 1935–1943.
Patel, H.H., Murray, F., Insel, P.A., 2008. G-protein-coupled receptor-signaling components in membrane raft and caveolae microdomains. Handb. Exp. Pharmacol. 186, 167–184.
Reed, D.R., Nanthakumar, E., North, M., Bell, C., Bartoshuk, L.M., Price, R.A., 1999. Localization of a gene for bitter-taste perception to human chromosome 5p15. Am. J. Hum. Genet. 64, 1478–1480.
Simons, K., Ehehalt, R., 2002. Cholesterol, lipid rafts, and disease. J. Clin. Invest. 110, 597–603.
Simons, K., Toomre, D., 2000. Lipid rafts and signal transduction. Nat. Rev. Mol. Cell Biol. 1, 31–39.
Stella, V.J., Rajewski, R.A., 1997. Cyclodextrins: their future in drug formulation and delivery. Pharm. Res. 14, 556–567.
Szejtli, J., Szente, L., 2005. Elimination of bitter, disgusting tastes of drugs and foods by cyclodextrins. Eur. J. Pharm. Biopharm. 61, 115–125.
Takagi, S., Toko, K., Wada, K., Yamada, H., Toyoshima, K., 1998. Detection of suppression of bitterness by sweet substance using a multichannel taste sensor. J. Pharm. Sci. 87, 552–555.
Toko, K., 1998. Electronic tongue. Biosens. Bioelectron. 13, 701–709.
Tsuruoka, M., Hashimoto, T., Seo, H., Ichimasa, S., Ueno, O., Fujinaga, T., Otagiri, M., Uekama, K., 1981. Enhanced bioavailability of phenytoin by β-cyclodextrin complexation. Yakugaku Zasshi 101, 360–367.
Uchida, T., Kobayashi, Y., Miyanaga, Y., Toukubo, R., Ikezaki, H., Taniguchi, A., Nishikata, M., Matsuyama, K., 2001. A new method for evaluating the bitterness of medicines by semi-continuous measurement of adsorption using a taste sensor. Chem. Pharm. Bull. 49, 1336–1339.
Uchida, T., Tanigake, A., Miyanaga, Y., Matsuyama, K., Kunitomo, M., Kobayashi, Y., Ikezaki, H., Taniguchi, A., 2003. Evaluation of the bitterness of antibiotics using a taste sensor. J. Pharm. Pharmacol. 55, 1479–1485.
Uekama, K., 2004. Design and evaluation of cyclodextrin-based drug formulation. Chem. Pharm. Bull. 52, 900–915.
Uekama, K., Hirayama, F., Irie, T., 1998. Cyclodextrin drug carrier systems. Chem. Rev. 98, 2045–2076.
Wong, G.T., Gannon, K.S., Margolskee, R.F., 1996. Transduction of bitter and sweet taste by gustducin. Nature 381, 796–800.
Zhang, Y., Hoon, M.A., Chandrashekar, J., Mueller, K.L., Cook, B., Wu, D., Zuker, C.S., Ryba, N.J., 2003. Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 112, 293–301.