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Biopharmaceutical Study of Inclusion Complexes. I. Pharmaceutical Advantages of Cyclodextrin Complexes of Bencyclane Fumarate[1)]

KEIJI FUJIOKA,* YUJI KUROSAKI, SHIGEJI SATO, TETSUO NOGUCHI, TAKESHI NOGUCHI and YOSHIYA YAMAHIRA

Formulation Research Department, Pharmaceutical Division, Sumitomo Chemical Co., Ltd., Kurakakiuchi 1–3–45, Ibaraki, Osaka 567, Japan

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Complexes of bencyclane fumarate (Ben) with cyclodextrins (CDs) were newly prepared and their characteristics were studied from a pharmaceutical viewpoint. The results of differential scanning calorimetry (DSC), X-ray diffractometry and thin-layer chromatography (TLC) were consistent with the formation of inclusion complexes. Water solubility of Ben–CDs were 2- to 8-fold lager than that of Ben. The stability of Ben in acidic media (pH 1.2) was considerably improved by complex formation with β-CD or γ-CD. Apparent first-order rate constants [h^{-1}] of hydrolysis of Ben were 6.5×10^{-2}, 5.5×10^{-2}, 1.0×10^{-2} and 1.7×10^{-2} for Ben, Ben–α-CD, Ben–β-CD and Ben–γ-CD, respectively. The intrinsic bitter taste of Ben was significantly reduced by inclusion complexation with CDs. Thus, there are clear pharmaceutical advantages of Ben–CD compared with Ben. In addition, a novel animal test method, which is helpful for studying astringent bitter-tasting drugs, is proposed.

Keywords——bencyclane fumarate; cyclodextrin; inclusion complex; stability; bitterness

Cyclodextrins (CDs) are well known to form inclusion complexes with various guest molecules.[2)] Inclusion complexes with CDs have been widely utilized as models for investigating enzymatic reactions.[3)] Recently, their application to drug delivery systems has been attempted in the pharmaceutical field.[4)] There are many reports which describe improvements of pharmaceutically unfavorable properties of a guest drug, such as solubilization of a poorly water-soluble drug,[5)] stabilization of a labile drug,[6)] reduction of irritancy[7)] or unpleasant odor,[8)] entrapment of a volatile drug,[9)] and so on by means of the formation of inclusion complexes.

Bencyclane, 3-[(1-benzylcycloheptyl)oxy]-*N*,*N*-dimethylpropylamine, developed by Pallos *et al.*[10)] has excellent anti-convulsant and vasodilative activities[11)] and has been supplied in the form of its fumaric salt (bencyclane fumarate, Ben). Ben has been used clinically for improvement of syndromes accompanying the obstruction of brain blood flow. On the other hand, Ben has some drawbacks such as instability in the acidic region[12)] and an intolerable bitter taste.

From a pharmaceutical viewpoint, it is possible that these intrinsic unfavorable properties of Ben might be improved by formation of inclusion complexes with CDs. However, no work on this has yet been done.

In this paper, the formation of inclusion complexes of Ben with CDs is described and their characteristics of pharmaceutical interest are presented. In addition, the reduction of bitterness by such complexation was comparatively estimated in human subjects and by a newly developed animal test method.

Experimental

Materials——Ben was obtained from Medimpex (Hungary). β-Cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD)

were donated by Nihon Shokuhin Kako Co. (Japan). α-Cyclodextrin (α-CD) was purchased from Nakarai Chemicals Co. (Japan). Cyclodextrins (CDs) were used without further purification. Other materials employed were of reagent grade.

Preparation of Ben–CD——Inclusion complexes of Ben with equimolar CD were prepared by two methods, as outlined in Chart 1.

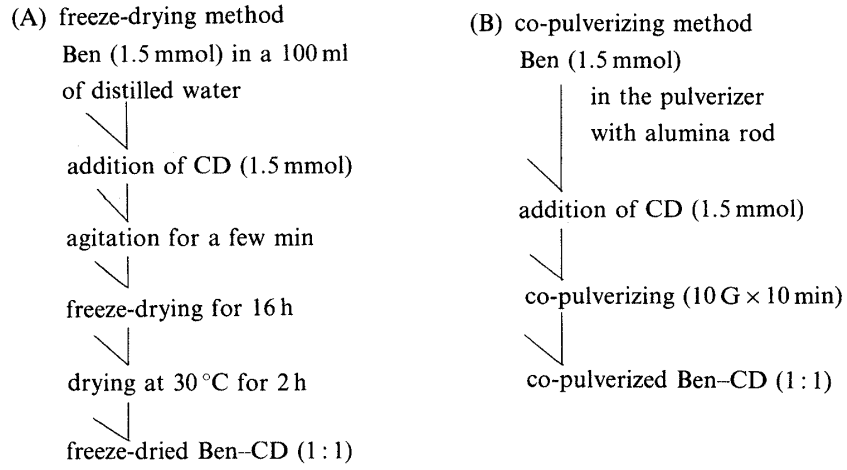


Chart 1. Method for the Preparation of Inclusion Complexes of Ben with CDs

Assay of Ben——Ben was assayed by gas-liquid chromatography (GLC). Ben was extracted from aqueous solution with chloroform under strongly alkaline conditions. The chloroform solution was then evaporated, and the residue was resolved with chloroform containing carbetapentane citrate as an internal standard. The solution was subjected to GLC under the following conditions. A model GC-5A apparatus (Shimadzu, Japan) equipped with a flame-ionization detector was used. The column was a 1.0 m × 3 mm glass tube packed with 5% APL on Chrom W. The temperature was maintained at 200 °C for the column and 220 °C for the injection port and the detector block. The flow rate of carrier gas (N_2) was set at 60 ml/min.

Determination of Water Solubility of Ben——Distilled water was employed. Ben or Ben–CD was vigorously shaken with water in a TS-type shaker (Tokiwa Seisakusho, Japan) at 120 strokes/min in the presence of undissolved Ben or Ben–CD for 30 min at 25 °C. After centrifugation at 2000 rpm for 10 min, the supernatant was collected and the concentration of Ben was determined.

Differential Sanning Calorimetry (DSC)——DSC was carried out with a differential scanning calorimeter (DSC-1B, Perkin-Elmer) at a scanning speed of 5 °K/min under an N_2 stream.

X-Ray Diffraction Analysis——Powder X-ray diffractometry was carried out under the following conditions using an X-ray diffractometer (Rigaku Geigerflex 2025). Conditions: target, Ni; voltage, 30 kV; divergency, 1 °C; receiving slit, 0.15 mm.

Thin-Layer Chromatography (TLC)——Precoated plates of Silica Gel G (Merck) 0.2 mm thick were used. A 15 μl aliquot of an aqueous solution corresponding to 1 mg/ml of Ben was applied to the chromatoplate. The developing solvent of the chromatogram was cyclohexane–isopropanol–ammonium hydroxide (28%) (80 : 20 : 1) and the spots were detected by exposure to iodine vapor.

Determination of the Stability in Aqueous Solution——Ben–CD corresponding to 200 mg of Ben was dissolved in 100 ml of 0.1 N HCl solution. The solution was maintained at 37 °C with gentle shaking and a 1 ml aliquot was sampled at predetermined times. Each specimen was assayed for remaining Ben by GLC.

Estimation of Bitterness——In this paper, the concentration of Ben–CDs is given as Ben-equivalent. Ben–CDs prepared by the freeze-drying method or acrinol as a reference were dissolved in distilled water at appropriate concentrations to make sample solutions. A 0.01% (w/v) solution of phenylthiocarbamide (phenylthiourea, PTC) was used as the standard bitter substance.[13] The relative bitterness of the test solution was estimated as follows. Five healthy male volunteers took part in the study. A 50 μl aliquot of the standard (PTC) or the sample solution was pipetted onto the examinee's tongue using a microsyringe. The examinee tasted the standard and sample solutions in turns in a blind manner and noted the relative bitterness on a previously prepared mark sheet. Data on the mark sheets of the five volunteers were then collected and the concentration of sample solution (X) that corresponded best to the bitterness of the standard was specified.

The Rabbit Eye-Blinking Test——Ben and Ben–CDs were dissolved in isotonic saline at various concentrations. Likewise acrinol as a reference was dissolved in isotonic phosphate buffer (pH 8.2) at various concentrations. Isotonic saline and isotonic phosphate buffer (pH 8.2) were employed as controls. Two drops (about 50 μl) of the sample

solution was put onto the cornea of a male albino rabbit of 2.5—3.0 kg body weight using Transpets® (Clay Adams). The response of the rabbit's eye was observed for one minute and the blinking count was recorded. The minimum concentration of each sample (Y) at which the blinking count significantly ($p<0.001$) exceeded that of the control was determined.

Results and Discussion

Water Solubility of Ben with CDs

There are many reports which describe an increase[14] or decrease[15] of water solubility of the guest molecule by formation of inclusion complexes. Table I summarizes the water solubility of equimolar complexes of Ben and CDs. Compared with Ben alone, complex formation with α-, β- and γ-CD by the freeze-drying method increased the warer solubility of Ben about 2-, 5.5- and 8-fold, respectively.

Recently the formation of inclusion complexes of some drugs with CDs by co-pulverization has been discussed.[16] Thus the effect of preparation method on the water solubility was examined in the case of Ben–β-CD. As shown in Table I, the water solubility of the complex prepared by the co-pulverizing method (see Chart 1) was nearly identical with that of the complex prepared by the freeze-drying method.

Physicochemical Properties of Ben–CD

In order to characterize the complexes of Ben with CDs, preliminary examinations by

Table I. Solubility of Ben–CDs in Water at 25 °C

Compound[a]	Preparation method[b]	Solubility (mg/ml)[c]
Ben		9.7
Ben–α-CD	F.D.	24.6
Ben–β-CD	F.D.	63.7
Ben–β-CD	C.P.	62.9
Ben–γ-CD	F.D.	78.6

a) CDs are equimolar to Ben.
b) Complexes were prepared by the freeze-drying method (F.D.) or the co-pulverizing method (C.P.).
c) This value represents Ben concentration. Each value is the mean of duplicate data.

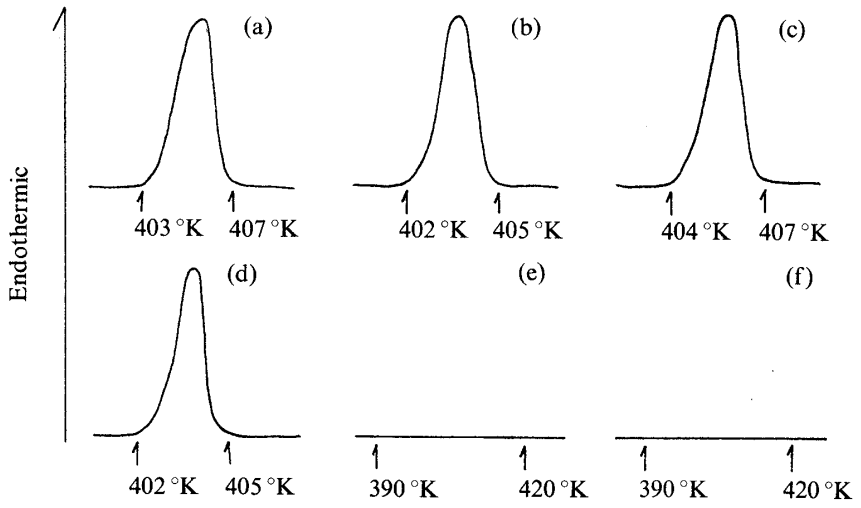


Fig. 1. DSC Curves of Ben/β-CD at a Scanning Speed of 5 °K/min

(a), intact Ben; (b), freeze-dreid Ben; (c), pulverized Ben; (d), sample made by adding freeze-dried β-CD to freeze-dried Ben equimolarly; (e), freeze-dried Ben–β-CD; (f) co-pulverized Ben–β-CD.

DSC, X-ray diffractometry and TLC were performed.

DSC curves of Ben and Ben–β-CD are shown in Fig. 1. An endothermic peak was observed around 404 °K for intact Ben (a), freeze-dried Ben (b), pulverized Ben (c) and for the mixture of freeze-dried Ben with freeze-dried β-CD (d). However, this peak disappeared completely in both freeze-dried Ben–β-CD (e) and co-pulverized Ben–β-CD (f). This disappearance of the endothermic peak was similarly observed for Ben–α- and Ben–γ-CD.

The X-ray diffraction patterns shown in Fig. 2 indicate that Ben, which was originally in a crystalline form, is apparently transformed to an amorphous state by the complex formation with CDs. This transformation did not occur on freeze-drying or pulverizing of Ben alone.

These results are consistent with the data of Kurozumi *et al.*[17] who described the preparation of inclusion complexes of non-steroidal antiinflammatory agents and other

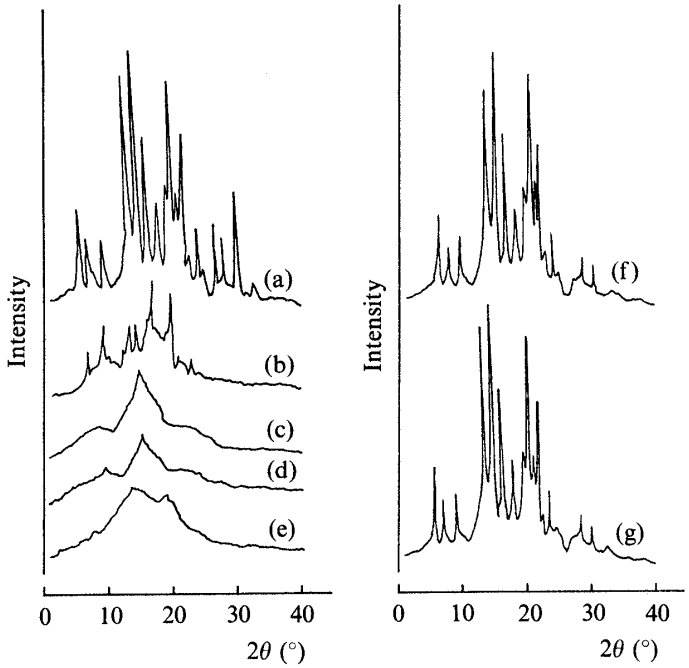


Fig. 2. X-Ray Diffraction Profiles of Ben/CDs

(a), Ben; (b), freeze-dried Ben–α-CD; (c), freeze-dried Ben–β-CD; (d), Co-pulverized Ben–β-CD; (e), freeze-dried Ben–γ-CD; (f), freeze-dried Ben; (g), pulverized Ben.

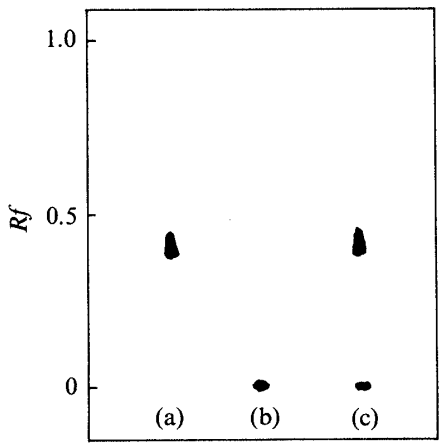


Fig. 3. Thin-Layer Chromatogram of Ben, β-CD and Ben–β-CD

(a), Ben; (b), β-CD; (c), Ben–β-CD. The developing solvent was cyclohexane–isopropanol–ammonium hydroxide (28%) (80:20:1). The spots were detected by exposing the plate to iodine vapor.

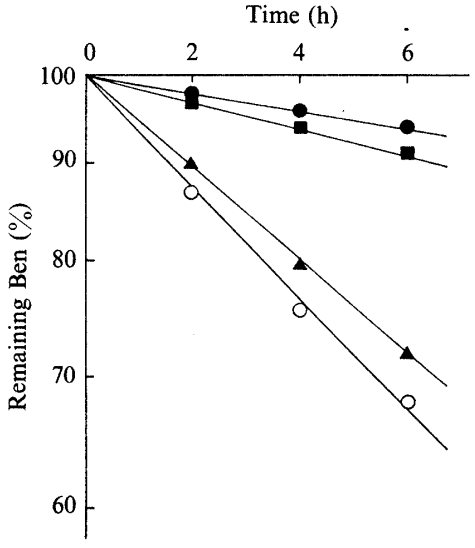


Fig. 4. Stability of Ben in 0.1 N HCl Solution at 37 °C

—○—, Ben; —▲—, Ben–α-CD; —●—, Ben–β-CD; —■—, Ben–γ-CD.

slightly water-soluble drugs with α- and β-CD.

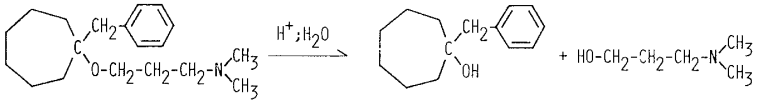
In a thin-layer chromatogram, Ben–β-CD separated completely to Ben and β-CD (Fig. 3) and no other spot suggesting a new compound was detected. Similar results were obtained in the cases of both Ben–α-CD and Ben–γ-CD. These results suggest that the complex formation of Ben–CD is a reversible phenomenon. All these results of DSC, X-ray diffractometry and TLC are consistent with inclusion complex phenomena so far reported,[18] and thus it was suggested that the interaction between Ben and CDs arose from inclusion complexation.

Stability of Ben–CD Complexes in Aqueous Solution

It has been reported that inclusion complexation of drugs with CDs affects the stability of guest drugs in aqueous solution; in some cases it delays the degradation[19] and in other cases, accelerates the decomposition.[20]

Figure 4 shows the stability of the freeze-dried Ben–CD complexes compared with that of Ben alone in 0.1 N HCl solution at 37 °C. In every case, the degradation of Ben seems to be kinetically an apparent monoexponential one. The degradation of Ben was delayed by complex formation with CDs. The delaying effect increased in the order of α-, γ- and β-CD. The apparent first-order rate constant (k_{app}) was estimated from the slope in Fig. 4. Estimated k_{app} [h^{-1}] values were 6.5×10^{-2}, 5.5×10^{-2}, 1.0×10^{-2} and 1.7×10^{-2} for Ben, Ben–α-CD, Ben–β-CD and Ben–γ-CD, and the calculated half-lives [h] were 10.7, 12.6, 69.3 and 40.8, respectively. It is thought that the difference in the delaying effect is partially attributable to the difference in the complex formation constants. For example, Ben–α-CD, which showed incomplete transformation to the amorphous state (see Fig. 2), had a smaller stabilizing effect. These results suggest that the complex formation constant of Ben–α-CD is much smaller than that of Ben–β-CD or Ben–γ-CD. In the case of co-pulverized Ben–β-CD, the degradation curve was completely in agreement with that of the freeze-dried Ben–β-CD (Fig. 4).

Simonyi *et al.*[12] demonstrated that Ben decomposed to cycloheptylbenzylcarbinol and dimethylaminopropanol by acidic hydrolysis (see Chart 2). Recently, Kigasawa *et al.*[21]



bencyclane — cycloheptyl-benzylcarbinol — dimethylamino propanol

Chart 2. Acid Hydrolysis of Bencyclane[12]

described further decomposition of cycloheptylbenzylcarbinol. Since the assay method employed in this paper measures the amount of Ben itself by gas chromatography, the hydrolysis of the ether linkage of Ben must be depressed by the complex formation, and thus access to this site is presumably sterically hindered by CDs.

Improvement of Taste of Ben by Inclusion Complexation

Ben has an astringent bitter taste which lasts for a few minutes. The reduction of this pharmaceutically unfavorable property by complex formation was estimated using PTC as the standard. PTC has been employed as a standard substance for bitterness in the study of bitter peptides.[13]

Table II shows the results of the estimation of relative bitterness. The concentrations at which the sample solutions corresponded to the standard 0.01% (w/v) PTC solution in bitterness were 0.2, 0.4, 2.0, 1.0 and 0.2% (w/v) for Ben, Ben–α-CD, Ben–β-CD, Ben–γ-CD and acrinol, respectively. Acrinol, selected as the common reference substance in this test and in the subsequent animal test, had a bitter taste nearly identical with that of Ben. The rank

order of the reducing effect on the bitter taste of Ben was Ben–β-CD > Ben–γ-CD ≫ Ben–α-CD and their relative bitterness ratios with respect to Ben were about 1/10, 1/5 and 1/2, respectively.

Although this estimation method, in which human volunteers taste the sample, is direct and reliable and is suitable for the purpose of formulation development, it can be burdensome to examinees, and is not always suitable for routine work.

Rabbit Eye-Blinking Test

To evaluate the bitter taste without using human examinees, a rabbit eye-blinking test was newly designed as described in the experimental section.

The results are shown in Table III. A clear dose dependence was obtained in the blinking count for Ben (or Ben–CDs) and acrinol. In the preliminary study, such a quantitative response was not clearly observed for PTC, quinine hydrochloride and caffeine, though these substances are known to be very bitter.[22] The difference between these substances and acrinol is probably due to the astringent bitterness of the latter.[22] Ben has similar bitterness to acrinol, and this kind of bitterness may be more suitable to be tested in the rabbit eye. The eye-blinking count may depend mainly on the irritation due to the astringent bitterness of the test solutions.

TABLE II. Estimation of Relative Bitterness in Human Volunteers

Conc. (%)	Ben	Ben–α-CD	Ben–β-CD	Ben–γ-Cd	Acrinol
0.05	−4[a]	−5	−5	−5	−5
0.1	−4	−5	−5	−5	−5
0.2	+3[b]	−3	−5	−5	+2[b]
0.4	+5	+2[b]	−5	−5	+5
0.7	ND[c]	+5	−4	−3	+5
1.0	ND	+5	−3	+2[b]	+5
2.0	ND	ND	+1[b]	+5	ND
4.0	ND	ND	+5	+5	ND

a) Each value is the sum of the points derived from the mark sheets. Scoring was as follows. +1; the sample solution is more bitter than 0.01% PTC, −1; the sample solution is less bitter than 0.01% PTC, 0; the sample solution and 0.01% PTC are nearly equal in bitterness.
b) The concentration of the sample solution (X) that corresponds most closely in bitterness to 0.01% PTC.
c) Not done.

TABLE III. Results of the Rabbit Eye-Blinking Test

Conc. (%)	Ben	Ben–α-CD	Ben–β-CD	Ben–γ-CD	Acrinol
0	1.4±0.9[a]				1.4±0.7[b]
0.001	1.8±0.9	1.7±0.8	ND[c]	ND	2.0±1.0
0.01	1.7±0.8	2.2±0.4	ND	ND	2.1±0.8
0.05	3.1±0.9[d]	1.8±0.6	ND	ND	2.3±0.9
0.1	3.6±1.0[d]	3.0±0.7[d]	1.1±0.6	2.0±0.7	4.5±1.2[e]
0.2	5.7±1.2[d]	3.7±0.8[d]	1.0±0.7	2.1±0.6	6.3±1.7[e]
1.0	ND	ND	1.1±0.6	3.5±0.5[d]	10.0±3.1[e]
2.0	ND	ND	1.6±1.5	9.2±2.8[d]	ND
4.0	ND	ND	7.3±1.4[d]	ND	ND

Each value is the mean blinking count±S.D. of eight experiments.
a) Control for Ben (–CD); 0.9% NaCl solution.
b) Control for acrinol; isotonic phosphate buffer (pH 8.2).
c) Not done.
d) Significantly different from control; *a*) ($p<0.001$).
e) Significantly different from control; *b*) ($p<0.001$).

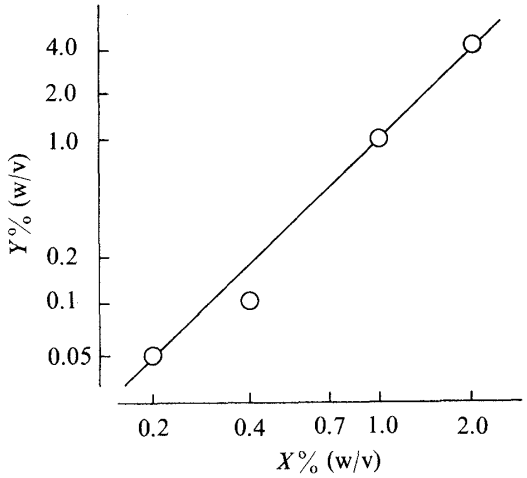


Fig. 5. Correlation between Human Tasting Test and Rabbit Eye-Blinking Test

X : Ben (equivalence) concentration at which Ben (–CDs) showed equivalent bitterness to 0.01% (w/v) PTC solution in human tasting test.

Y : The minimum concentration of Ben (equivalence) at which the blinking count significantly ($p<0.001$) exceeded that of 0.9% NaCl solution (control) in the rabbit eye-blinking test.

As shown in Table III, the minimum concentrations (Y) at which the blinking count significantly exceeded that of the control were 0.05, 0.1, 4.0 and 1.0% (w/v) for Ben, Ben–α-CD, Ben–β-CD and Ben–γ-CD, respectively, and 0.1% (w/v) for acrinol. The irritativity of Ben to the rabbit's eye was significantly reduced when Ben formed inclusion complexes with CDs. The rank order of the reducing effect on the irritativity to rabbit's eye was Ben–β-CD > Ben–γ-CD ≫ Ben–α-CD and their relative irritativities with respect to Ben were about 1/80, 1/20 and 1/2, respectively.

Correlation between Estimations in Man and in the Rabbit

The correlation between bitterness in the human study and irritativity to the rabbit's eye was examined. The concentrations at which Ben or Ben–CDs showed equivalent bitterness to 0.01% (w/v) PTC solution (X) and the minimum concentrations at which the blinking count significantly exceeded the control in the rabbit eye-blinking test (Y) were plotted on a log–log scale (Fig. 5). A regression line was calculated according to the following equation.

$$\ln Y = A(\ln X) + B$$

Values of $A = 1.99$ and $B = -0.06$ were obtained. The value B, which represents the discrepancy constant between the two tests, is very small, and a good regression coefficient of $r = 0.99$ was found between ln (X) and ln (Y).

Thus it was clearly demonstrated that the rabbit study can be employed as a substitute for human testing. Moreover, as the rabbit eye-blinking test showed wider effective concentration range than human tasting ($A = 1.99$), differences in the strength of the astringent bitterness can be detected more evidently in the animal test method. There has been little previous work on the quantitative evaluation of bitterness of drugs, and the method presented here is a novel one for evaluating bitter taste.

From the above-mentioned results, it is concluded that reversible interaction, which was proved to be inclusion complexation, readily occurred between Ben and CDs during simple procedures such as freeze-drying or co-pulverizing. Moreover, in the cases of Ben–β-CD and Ben–γ-CD, the water solubility, the stability in acidic media and the undesirable taste of Ben were markedly improved.

The influence of inclusion complexation on the characteristics of the guest molecule is considered to be affected by both the site of the inclusion, *i.e.*, the structure of the complex, and the strength of the complex formation, *i.e.*, the complex formation constant. The apparent rate constants of acidic hydrolysis (k_{app}) were in the order of Ben > Ben–α-CD ≫ Ben–γ-CD > Ben–β-CD. Likewise, both the strength of bitter taste in humans and the irritativity to the rabbit's eye were in the order of Ben > Ben–α-CD ≫ Ben–γ-CD > Ben–β-

CD. These results suggest that those three factors are similarly influenced by the complex formation and are closely related.

Among Ben–CDs, Ben–β-CD seems to be the most preferable complex from the viewpoints of both stability in acidic media and the taste. In this work, solid complex was used for all experiments because a simple mixture powder of Ben and CD exhibited bitter astringent taste in man in the preliminary study. In the case of practical formulation of solid dosage forms, taste is one of the important considerations. Thus, it may be possible to obtain a superior preparation of Ben by the application of Ben–β-CD.

References and Notes

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