γ-Cyclodextrin as Inhibitor of the Precipitation Reaction between Berberine and Glycyrrhizin in Decoctions of Natural Medicines: Interaction Studies of Cyclodextrins with Glycyrrhizin and Glycyrrhetic Acid by ^{1}H-NMR Spectroscopy and Molecular-Dynamics Calculation

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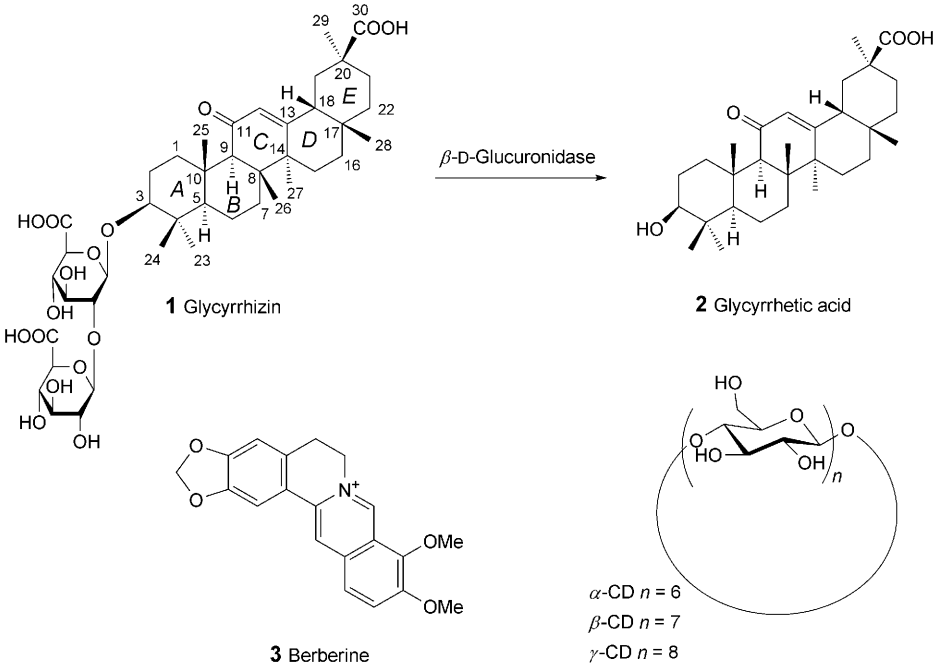
To prevent the precipitation reaction between glycyrrhizin (**1**) and berberine (**3**) in the decoctions of *Glycyrrhiza*/*Coptis* rhizome or *Glycyrrhiza*/*Phellodendron* bark, the presence of cyclodextrin (CD) in the mixture was proven to be effective. The preventing effect decreased in the order γ-CD > β-CD, and no effect was observed for α-CD. On the other hand, the extraction degree of **1** from the natural medicine *Glycyrrhia* was considerably increased in the presence of γ-CD, γ-CD being much more effective than α- or β-CD. Thus, the blocking effect of CD on the precipitate formation between **1** and **3** is suggested to be primarily dependent on the stability of the inclusion complex of the CD with **1**. To establish the structure of such a preferred inclusion complex, the interactions of **1** with β- and γ-CDs were investigated by ^{1}H-NMR spectroscopy and molecular-dynamics (MD) calculations. The ^{1}H-NMR measurements showed that the increase in solubility of **1** in H_2O is dependent on the degree of its inclusion into the CD, which depends on the molecular size of the CD. The MD calculations suggested that the H-bond interactions are sufficiently strong to form a stable [**1**/γ-CD] complex, in which the lipophilic rings C, D, and E of **1** are fully inserted into the molecular cavity of γ-CD, thus forming a kind of structure covered by a hydrophilic molecular capsule, while such an interaction mode is impossible for α- or β-CD.

Introduction. – *Glycyrrhiza* [1] (*Glycyrrhiza uralensis* FISHER and *G. glabra* LINNE (Leguminosae)) has been mostly used as a Chinese crude medicine (Kampo). In addition to the sweet-tasting effect allowing to take Kampo easily, *Glycyrrhiza* contains some components exhibiting pharmacological activity. The main substance of the sweetness is glycyrrhizin (**1**; see *Scheme 1*) [2][3] which is an oleanane-type saponin and about 150 times sweeter than sucrose. Glycyrrhizin (**1**) also exhibits mineralocorticoid and anti-inflammatory activity, and various other pharmacological activities [4–6]. Glycyrrhetic acid (**2**) [7], the sapogenin of **1**, formed on hydrolysis by β-D-glucuronidase (*Scheme 1*), also shows pharmacological activities [8]. Because **1** is difficult to be directly absorbed from the digestive tract, the main active body in the oral administration of *Glycyrrhiza* extracts could be **2**; **2** has been confirmed to be the active component responsible for the hepatic-disorder inhibitory effect and the hepatocyte-failure protective action on taking **1**. In the Japanese Pharmacopoeia, the following rule is regulated: the *Glycyrrhiza* should contain more than 2.5% of **1** [1]. Thus, to exhibit the medicinal effects, the content of **1** should be above this value in the extraction liquid of the natural medicine.

Scheme 1



Noguchi and co-workers reported [9–12] that the alkaloid component berberine (**3**) in the crude drug *Coptis* rhizome forms an insoluble complex through an ionic interaction with another component, glycyrrhizin (**1**) in *Glycyrrhiza*, and consequently the content of **3** in the decoction of *Coptis* rhizome/*Glycyrrhiza* decreases by *ca.* 30%, as compared with that of *Glycyrrhiza*-free *Coptis* rhizome. It is important to find a method for preventing the loss of the active components in multiple crude drugs. On the other hand, we showed that the solubility of alkaloids such as **3** [13] and ephedrin [14] in water was increased in the presence of cyclodextrins (CDs), depending on the size of the cyclic oligosaccharide consisting of 6 (α-CD), 7 (β-CD), or 8 (γ-CD) glucose units. Although these CDs have similar physicochemical properties, the potential to form inclusion complexes with a guest molecule is highly dependent on the size of the inclusion cavity of each CD [15].

Against these backgrounds, we investigated the inhibitory effect of α-, β-, and γ-CD on the ionic-complex formation between **1** and **3**, and the interaction effect of these CDs with **1** using ^{1}H-NMR spectroscopy and molecular-dynamics (MD) calculations, to confirm the structural scaffold of γ-CD which allowed to increase the solubility of **1** and **2** in water.

Experimental. 1. *Materials.* Glycyrrhizin ((=(3β,20β)-20-carboxy-11-oxo-30-norolean-12-en-3-yl 2-*O*-β-D-glucopyranuronosyl-β-D-glucopyranosiduronic acid; **1**), glycyrrhetic acid (=(3β,20β)-3-hydroxy-11-oxoolean-12-en-30-oic acid; **2**), berberine (=5,6-dihydro-9,10-dimethoxybenzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizinium; **3**), and α-, β-, and γ-cyclodextrin (α-, β-, and γ-CD) were purchased from

Nacalai Tesque Inc. (Kyoto), and used after recrystallization from their aq. soln. The dried and cut crude-drug natural medicines *Coptis* rhizome (*Coptis japonica, C. chinensis, C. deltoide*, and *C. teeta* (Ranunculaceae)), *Phellodendron* bark (*Phellodendron amurense* and *P. chinense* (Rutaceae)), and *Glycyrrhiza* (*Glycyrrhiza uralensis* FISHER and *G. glabra* LINNE (Leguminosae)) were purchased from *Tochimoto Tenkaido Co. Ltd.* (Osaka).

2. *Extraction of* **1** *and* **3** *from the Mixtures of the Natural Medicines* Glycyrrhiza *and* Coptis *Rhizome or* Phellodendron *bark.* The α-, β-, or γ-CD (500 mg, 12.5% with respect to the natural-medicine mixture) was added to the test tube containing the natural medicine *Glycyrrhiza* powder (2 g) and *Coptis* rhizome (2 g) or *Phellodendron* bark (2 g) in H_2O (40 ml), and was heated at 100° for 2 h in a metal bath. The extract was cooled with standing at 24° for 1 h, and the supernatant was filtrated (0.45 μm filter size; *Millex-HA*, *Millipore Co.*). The quantity of **1** and **3** in the filtrate was determined by HPLC.

3. *HPLC Analysis of* **1** *and* **3** (see below, *Fig. 1*). *Waters* HPLC system: pump, *Waters-600* controller, 0.8 ml/min; detector, *Waters 996 PDA* (**1**: detection at 254 nm; **3**: detection at 345 nm); column, *Cosmosil 5C18-AR-II*, 4.6 mm i.d. × 150 mm (*Nacalai Tesque*). Detection conditions of **1** and **3**: mobile phase for **1**, 6.67% AcOH/MeCN 60 : 40); mobile phase for **3**, 0.17% sodium lauryl sulfate, 0.34% KH_2PO_4, and 50% MeCN in H_2O; each sample (20 μl) was injected 5 times; calculations with the Empower 2 software (by the average peak area). The peak-area values are given rel. to the peak area of **1** extracted from *Glycyrrhiza* and **3** extracted from *Coptis* rhizome and *Phellodendron* bark, resp. The peak-area values (in parentheses) of **1** and **3** in the presence of α-, β-, or γ-CD were as follows: in the decoction of *Glycyrrhiza* (**1**: t_R 5.88, peak area 100%), of *Coptis* rhizome (**3**: t_R 11.54, peak area 100%), of *Glycyrrhiza*/*Coptis* rhizome (**1**: t_R 5.96, peak area 31%; **3**: t_R 11.98, peak area 38%), of *Glycyrrhiza*/*Coptis* rhizome/α-CD (**1**: t_R 6.20, peak area 29%; **3**: t_R 11.65, peak area 30%), of *Glycyrrhiza*/*Coptis* rhizome/β-CD (**1**: t_R 6.13, peak area 49%; **3**: t_R 11.87, peak area 37%, of *Glycyrrhiza*/*Coptis* rhizome/γ-CD (**1**: t_R 6.08, peak area 101%; **3**: t_R 11.62, peak area 80%), of *Phellodendron* bark (**3**: t_R 11.82, peak area 100%), of *Glycyrrhiza* (**1**: t_R 6.12, peak area 100%), of *Glycyrrhiza*/*Phellodendron* bark (**1**: t_R 6.10, peak area 41%; **3**: t_R 12.74, peak area 40%), of *Glycyrrhiza*/*Phellodendron* bark/α-CD (**1**: t_R 6.15, peak area 43%; **3**: t_R 12.31, peak area 44%), of *Glycyrrhiza*/*Phellodendron* bark/β-CD (**1**: t_R 6.14, peak area 58%; **3**: t_R 12.94, peak area 41%), and of *Glycyrrhiza*/*Phellodendron* bark/γ-CD (**1**: t_R 6.13, peak area 99%; **3**: t_R 12.84, peak area 64%).

4. *Solubility of* **1** *and* **2** *in* H_2O. Compound **1** or **2** (5 mg) was dissolved in H_2O (3.5 ml) with a known quantity of α-, β-, or γ-CD (see below, *Fig. 2*) at 80° for 30 min and then further incubated for 2 h at 24°. After the incubation, the mixture was filtered (0.45 μm filter size; *Millex-HA*, *Millipore Co.*), and the content of **1** or **2** was determined by HPLC and UV.

5. *Extraction of* **1** *from the Natural Medicine* Glycyrrhiza (see below, *Fig. 3*). The α-, β-, or γ-CD (100 mg, 5% with respect to the natural medicine) was added to the test tube containing the natural medicine *Glycyrrhiza* powder (2 g) in H_2O (10 ml), and was heated at 80° for 0.5 h in a metal bath. The extract was cooled with standing at 24° for 1 h, and the supernatant was filtrated (0.45 μm filter size; *Millex-HA*, *Millipore Co.*). The quantity of **1** in the filtrate was determined by HPLC (*cf. Sect. 3*, except for flow rate 1.0 ml/min, detector *Waters 2487* (UV detector 254 nm), and mobile phase H_2O/MeCN 3 : 2.

6. *^{1}H-NMR Measurement. Varian-Inova-500* spectrometer (^{1}H at 497.2 MHz) at 26°; δ in ppm rel. to TSP (= sodium 3-(trimethylsilyl)(2,2,3,3-D_4)propanoate) as an external reference; concentration of **1** *ca.* 1 mM in D_2O solns. ^{1}H-NMR of **1** in the presence of α-, β-, or γ-CD: **1**/α-CD 1 : 0: 1.290 (*s*, Me(29)); 2.020 (*s*, H–C(18)); 2.400 (*s*, H–C(9)); 5.575 (*s*, H–C(12)); **1**/α-CD 1 : 0.05: 1.290 (*s*, Me(29)); 2.021 (*s*, H–C(18)); 2.400 (*s*, H–C(9)); 5.570 (*s*, H–C(12)); **1**/α-CD 1 : 0.1: 1.291 (*s*, Me(29)); 2.020 (*s*, H–C(18)); 2.399 (*s*, H–C(9)); 5.574 (*s*, H–C(12)); **1**/α-CD 1 : 0.15: 1.290 (*s*, Me(29)); 2.020 (*s*, H–C(18)); 2.399 (*s*, H–C(9)); 5.575 (*s*, H–C(12)); **1**/β-CD = 1 : 0.05: 1.310 (*s*, Me(29)); 2.035 (*s*, H–C(18)); 2.401 (*s*, H–C(9)); 5.595 (*s*, H–C(12)); **1**/β-CD 1 : 0.1: 1.315 (*s*, Me(29)); 2.040 (*s*, H–C(18)); 2.402 (*s*, H–C(9)); 5.606 (*s*, H–C(12)); **1**/β-CD 1 : 0.15: 1.318 (*s*, Me(29)); 2.041 (*s*, H–C(18)); 2.401 (*s*, H–C(9)); 5.615 (*s*, H–C(12)); **1**/γ-CD 1 : 0.05: 1.310 (*s*, Me(29)); 2.261 (*s*, H–C(18)); 2.750 (*s*, H–C(9)); 5.615 (*s*, H–C(12)); **1**/γ-CD 1 : 0.1: 1.342 (*s*, Me(29)); 2.421 (*s*, H–C(18)); 3.000 (*s*, H–C(9)); 5.675 (*s*, H–C(12)); **1**/γ-CD 1 : 0.15: 1.370 (*s*, Me(29)); 2.591 (*s*, H–C(18)); 3.101 (*s*, H–C(9)); 5.535 (*s*, H–C(12)).

7. *Molecular-Dynamics Calculations.* The atomic coordinates of β-CD used for the MD calculations were constructed from its X-ray crystal analysis [16], and the atomic coordinates of **1** and γ-CD were constructed by using Insight II/Discover 2000 [17] and Discovery Studio Modeling (*Accelrys*) [17]. By

taking the results of the ^{1}H-NMR experiments into consideration, **1** was positioned so as to allow the approach from the secondary-OH-group rim of β- or γ-CD as the starting structure for the MD calculation, wherein the distance between the O-atoms of CD and **1** is >5 Å. To simulate the aq. soln. system, 2969 H_2O molecules for β-CD or 5718 for γ-CD were added in a 6.0 Å-truncated octahedron-shaped cell. The CHARMm [18] in the Discovery Studio Modeling [17] program package was used for the MD simulation, where the force field used was from cff[1]), and the time step of 1 fs was taken for the simulation. The cut-off radius was set to 13.5 Å within the group base, and the simulation was carried out for 1 ns. By setting the periodic boundary conditions with an NVT ensemble, the MD simulations were performed while the final temp. was set to 300 K.

Results. – 1. *Effect of CDs on the Precipitation Reaction between* **1** *and* **3** *in the Decoction of Natural Medicines. Fig. 1* shows the HPLC yields of **1** and **3** in the decoctions of *Coptis* rhizome/*Glycyrrhiza* and *Phellodendron* bark/*Glycyrrhiza*. In the presence of *Glycyrrhiza*, the amount of **3** decreased to *ca.* 40% as compared with the amount in the decoction of *Coptis* rhizome alone (*Fig. 1,a*). However, when β- or γ-CD was added to *Coptis* rhizome/*Glycyrrhiza* in H_2O before extraction, the yield of **1** increased, and a recovery of *ca.* 100% was reached by the addition of γ-CD. A similar profile was also observed for *Phellodendron* bark/*Glycyrrhiza* (*Fig. 1,b*). Thus, these results showed that although the amount of extracted **1** or **3** in the decoctions of *Coptis* rhizome/*Glycyrrhiza* and *Phellodendron* bark/*Glycyrrhiza* was significantly decreased as compared to the decoctions obtained in the absence of *Glycyrrhiza*, the addition of CDs led to the recovery of **3** up to the values obtained in the absence of *Glycyrrhiza*. The recovery effect was best in the presence of γ-CD $>\beta$-CD for **1** and of γ-CD for **3** in the mixed crude drugs; α-CD had no effect on the extraction of **1** and **3**, and β-CD was inefficient for that of **3**.

2. *Solubility of Inclusion Complexes [***1***/CDs] and [***2***/CDs] in H_2O.* The increase of the solubility of **1** or **2** in H_2O in the presence of α-, β-, or γ-CD was investigated by HPLC. In the case of **1**, the solubility was significantly increased only in the presence of γ-CD (*Fig. 2,a*), the apparent increasing constant k being 2.6 mol/l at 24° under the experimental conditions. This is in contrast with the influence of α-CD or β-CD.

Similar, but more prominent effects were also observed for **2** (*Fig. 2,b*). γ-CD increased the solubility of **2** with a k value of 9.1 mol/l, whereas no notable effect on the solubility occurred by the addition of α-CD or β-CD.

3. *Extraction of* **1** *from the Crude Medicine* Glycyrrhiza. The effect of CDs on the extraction of **1** from the crude medicine *Glycyrrhiza* powder was examined because **1** and **2** were shown to interact with CDs in aqueous solution. The quantity of **1**, extracted from the H_2O used to infuse *Glycyrrhiza*, as determined by HPLC is shown in *Fig. 3*. The presence of γ-CD increased the extracted quantity of **1** remarkably, compared with that in the absence of CD or with that in the presence of α- or β-CD: the extraction effect on **1** of γ-CD (5% with respect to *Glycyrrhiza*) was 140% as compared with that without CD, whereas α- and β-CD were essentially ineffective.

4. *^{1}H-NMR Analysis.* To establish that the structural scaffold of γ-CD is responsible for the inhibition of the precipitation of **1** with **3** and the increase of the solubility of **1** in H_2O, the interaction mode between **1** and γ-CD in aqueous solution was studied by ^{1}H-NMR spectroscopy and compared with that between **1** and β-CD. It is generally

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[1]) Force field was automatically added by using cff in [19].

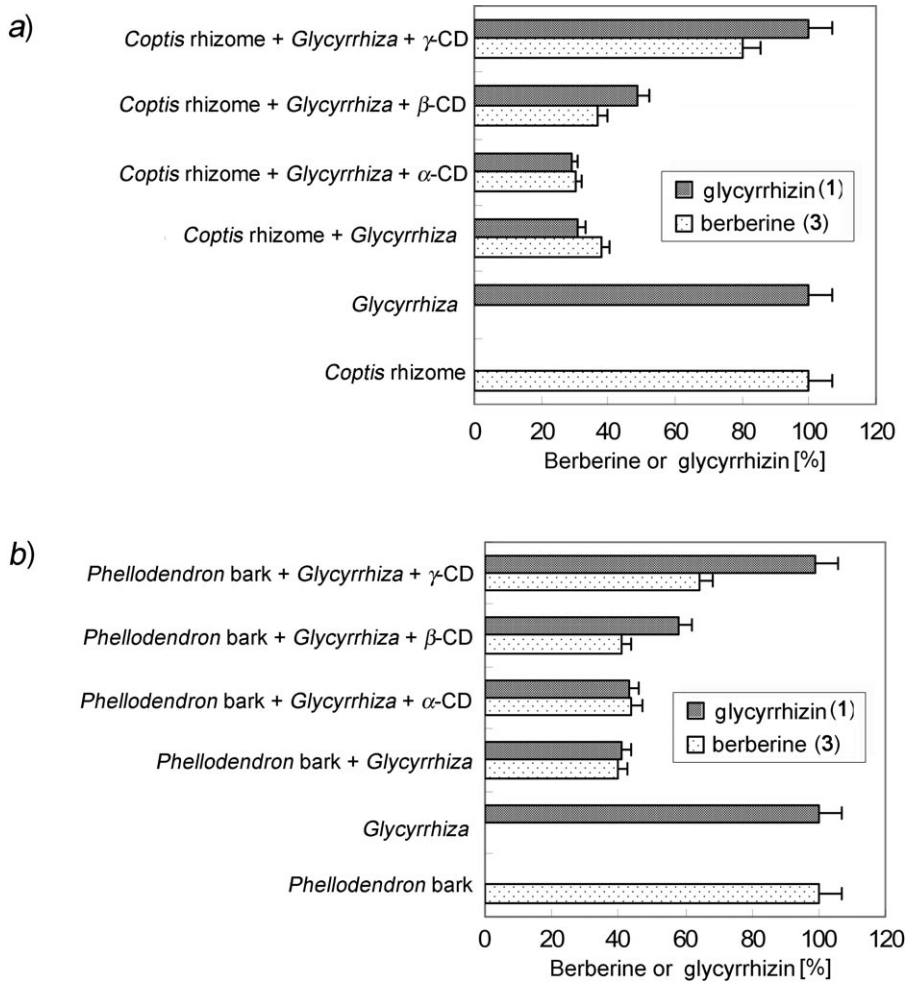


Fig. 1. *Yields of glycyrrhizin* (**1**) *and berberine* (**3**) *in the decoction of* a) Coptis *rhizome*/Glycyrrhiza *and* b) Phellodendron *bark*/Glycyrrhiza *after addition of α-, β-, or γ-CD, as determined by HPLC*

known that the δ(H) values of the guest molecule are shifted up- or downfield depending on the interaction mode, when the guest molecule inserts into the cavity of CD [20–22].

The chemical-shift change ($\Delta\delta$(H)) of each proton signal of **1** as a function of the γ-CD concentration is shown in *Fig. 4,a*. The signal of H–C(9) is most largely shifted downfield, and the $\Delta\delta$(H) toward lower field of the other protons decrease in the order H–C(18) > H–C(12) > Me–C(20), these lower-field shifts being obviously indicative of the interaction with γ-CD. As the concerned H-atoms are located at the rings C, D, and E of **1**, this suggests that the rings C, D, and E are inserted into the cavity of γ-CD (*Fig. 5*). On the other hand, in the presence of β-CD (*Fig. 4,b*), the $\Delta\delta$(H) of H–C(12), Me–C(20), and H–C(18) of **1** toward lower field were small and the signal

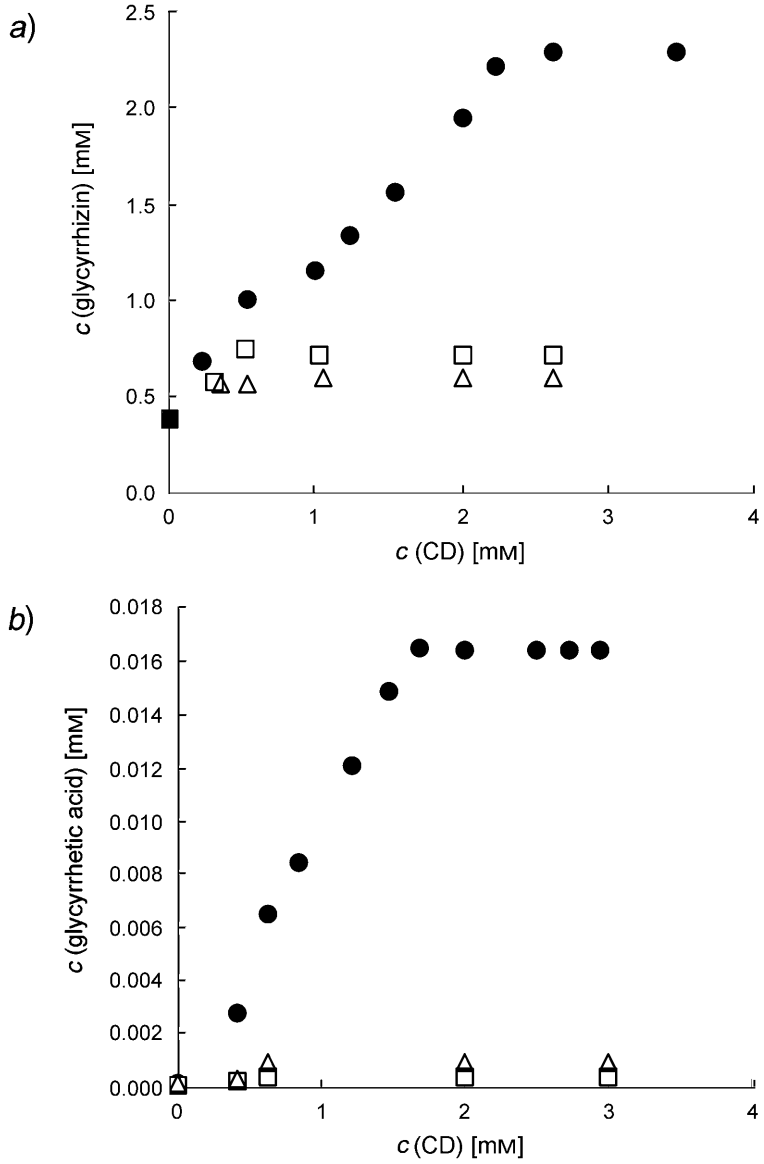


Fig. 2. *Change of cyclodextrin-dependent solubility of* a*) glycyrrhizin* (**1**) *and* b*) glycyrrhetic acid* (**2**) *in water at 24°.* □: α-CD; △: β-CD; ●: γ-CD.

of H–C(9) was almost unchanged, suggesting a partial insertion of H–C(18) and Me–C(20) located at ring E into the cavity of β-CD (*Fig. 5*). The δ(H) of **1** were unchanged in the presence of α-CD, suggesting no interaction between **1** and α-CD.

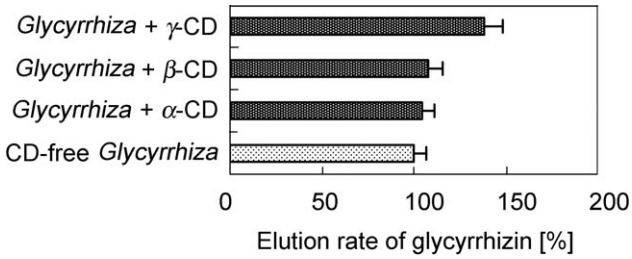


Fig. 3. *Increasing effect of CDs for extraction of glycyrrhizin* (**1**) *from the natural medicine* Glycyrrhiza

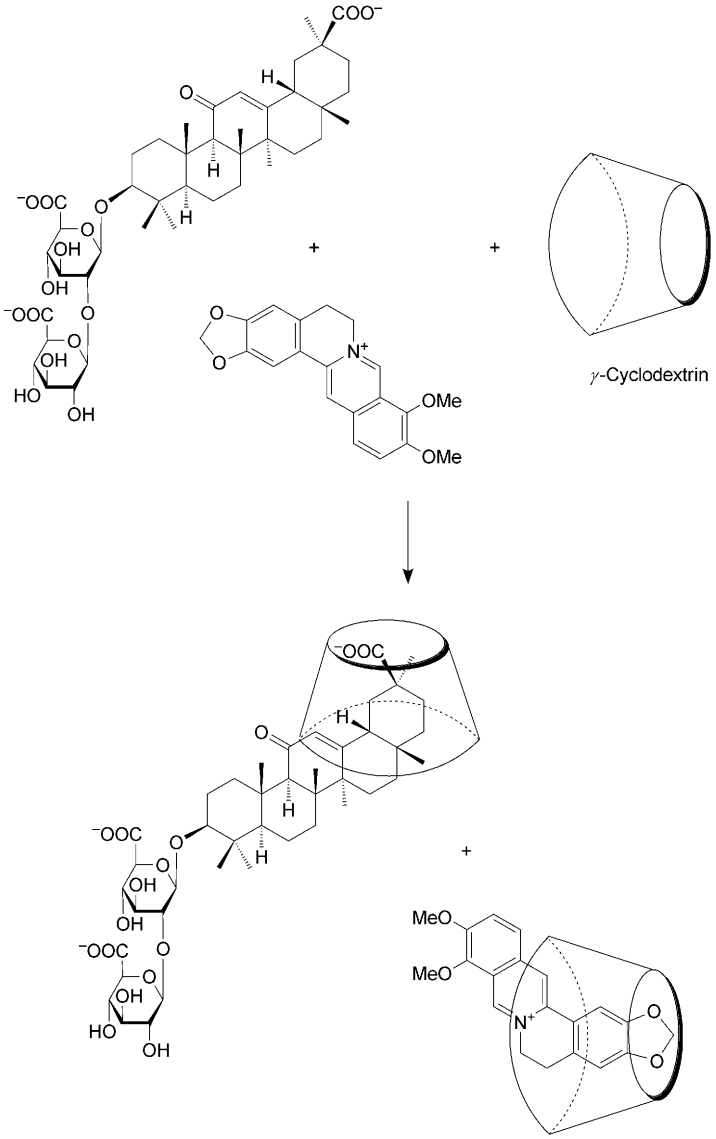
5. *Molecular-Dynamics Calculations.* To confirm that the structural scaffold of the CDs is responsible for the interactions as estimated from the ^{1}H-NMR analysis, the interactions of **1** with β-CD and γ-CD in aqueous solution were simulated by MD calculations. As for the [**1**/β-CD] complex, the MD simulation during 1 ns showed that **1** is fixed at the entrance of β-CD without penetration into or expulsion from the inside of β-CD, a state in which only ring E of **1** is completely inserted into the cavity of β-CD. This interaction mode does not disagree with the proton behavior observed by the NMR measurement (*Fig. 5*). On the other hand, concerning the interaction between **1** and γ-CD, in the case of the approach of **1** from the secondary-OH-group rim of γ-CD, the rings C, D, and E of **1** are fully inserted into the cavity of γ-CD, thus explaining well the proton behaviors established by the ^{1}H-NMR measurement (*Fig. 5*).

Discussion. – It was reported [12] that **3** in the *Coptis* rhizome forms the insoluble precipitate $[(\mathbf{3}^+)_{1\text{ or }2}-\mathbf{1}^-]$ in the presence of **1** from *Glycyrrhiza*, and the amount of soluble **3** decreases to about half in aqueous solution. The present HPLC results established the ability of γ-CD to inhibit the precipitation reaction between **1** and **3** in the mixed crude drugs. The inhibition of $[(\mathbf{3}^+)_{1\text{ or }2}-\mathbf{1}^-]$ precipitation by the addition of γ-CD would be as follow: When γ-CD is present in the solution of **1** and **3**, the inclusion complexes [**1**/γ-CD] and [**3**/γ-CD] [13] are preferentially formed, because their formation rate is much faster than that of the complex formation between **1** and **3**. An inhibition model for the precipitation reaction of **1** and **3** by γ-CD based on the present results is shown in *Scheme 2*. β-CD was also suitable for the inhibition of such a precipitation, although its efficiency was lower than that of γ-CD.

On the other hand, the addition of γ-CD increased the extraction quantity of **1** from its crude medicine *Glycyrrhiza* to *ca.* 140%, indicating that the solubility of **1** in aqueous solution was increased by the formation of the stable complex with γ-CD; the effect of α- and β-CDs on the extraction was not so significant.

To establish that the structural scaffold of γ-CD is responsible for the inhibition of the precipitation of **1** with **3** and the solubility increase of **1** in water, the anticipated complex structure [**1**/γ-CD] was investigated by ^{1}H-NMR measurements and MD calculations. In the presence of γ-CD, the chemical-shift values of the protons located at rings C, D, and E of **1** were significantly changed. This obviously suggested the insertion of the rings C, D, and E of **1** into the skeletal middle position of the γ-CD cavity. In contrast, the proton chemical shifts of **1** were not significantly changed by α-CD, and in the presence of β-CD, the ^{1}H-NMR signals of the protons at and near ring E of **1** were

Scheme 2. *Possible Interaction Model of* **1** *and* **3** *in the Decoction of* Coptis *Rhizome/Glycyrrhia or* Phellodendron *bark*/Glycyrrhiza *in the Presence of* γ*-CD*



slightly moved downfield, probably due to the partial interaction with the OH groups of β-CD.

The MD calculations proposed a stable inclusion complex [**1**/γ-CD], and its interaction mode explained well the proton behaviors observed in the ^{1}H-NMR analysis. In the structure of [**1**/γ-CD] at 488 ps in MD (*Fig. 6*), the O–C(30) at ring E

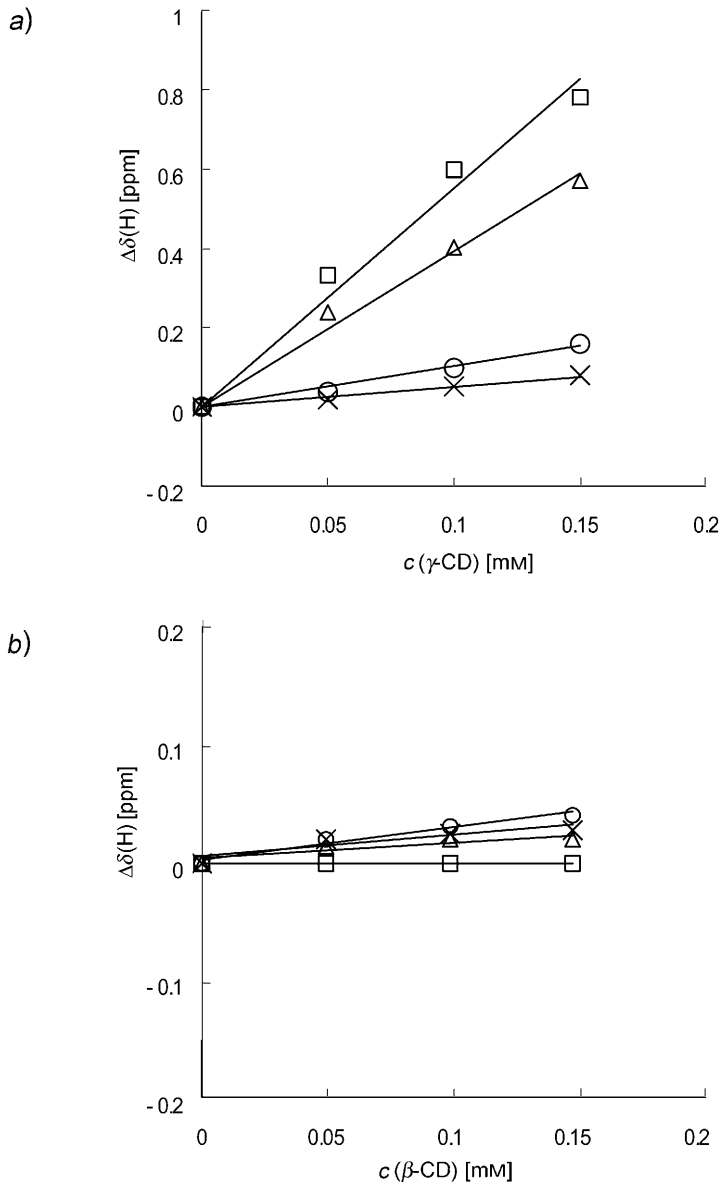


Fig. 4. *Chemical-shift change Δδ(H) of* **1** *in the presence of* a) *γ-CD and* b) *β-CD in* D_2O. *a*) ○: H−C(12) ($R^2 = 0.99$); □: H−C(9) ($R^2 = 0.97$); △: H−C(18) ($R^2 = 0.98$); ×: Me−C(20) ($R^2 = 0.99$). *b*) ○: H−C(12) ($R^2 = 0.96$); □: H−C(9); △: H−C(18) ($R^2 = 0.83$); ×: Me−C(20) ($R^2 = 0.83$).

and the O−C(11) at ring C of **1** were located close to the primary- and secondary-OH rim of *γ*-CD, respectively, thus indicating the stabilization of the complex through H-bonds, *i.e.*, *γ*-CD forms a kind of hydrophilic molecular capsule for **1**, leading to the precipitation inhibition and the increased extraction and solubility of **1**.

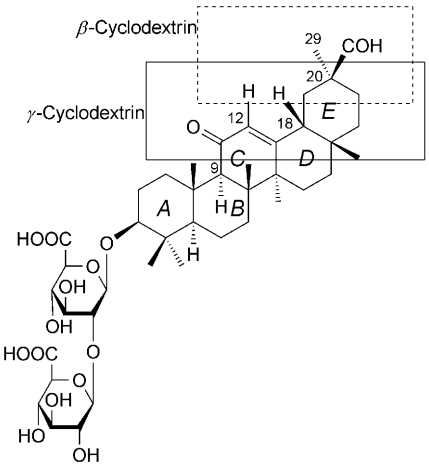


Fig. 5. *Moiety of* **1** *inserted into the cavity of γ-CD* (straight line) *and β-CD* (dotted line), *as estimated from the* ^{1}*H-NMR chemical-shift changes*

In recent years, the utilization of CDs for the effective extraction of lipophilic alkaloids from natural medicines has received much attention [14]. Our results suggest that the presence of CDs is not only efficient for the extraction of increased amounts of alkaloids but also of saponins from natural medicines, besides that some CDs are efficient inhibitors of the formation of insoluble precipitations arising from the reaction between the components in decoctions of natural medicines.

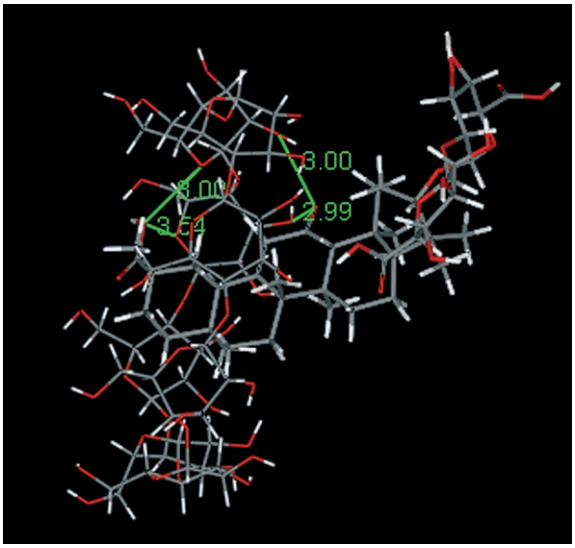


Fig. 6. *Snapshot of* the *[***1***/γ-CD] complex at 488 ps MD calculation*. The O–O atomic pairs between **1** and γ-CD are shown by green lines, together with their distances [Å], **1**: stick model; γ-CD: line model.

REFERENCES

[1] 'The Japanese Pharmacopoeia', 15th edn., Ministry of Health, Labour and Welfare, 2007, pp. 1185, 1187–1188, and 1197.
[2] C. Norman, *Chemisch2Weekblad* **1952**, *48*, 213.
[3] B. Lythgoe, S. Trippett, *J. Chem. Soc.* **1950**, 1983.
[4] T. Hattori, T. Nagamatsu, M. Ito, Y. Suzuki, *Jpn. J. Pharmacol*. **1989**, *51*, 117.
[5] A. Kumagai, M. Nanaboshi, Y. Asanuma, T. Yagura, K. Nishino, *Endocrinologia Japonica* **1967**, *14*, 39.
[6] H. Gerold, B. Lidia, M. Martin, K. Rimma, B. Lia, A. T. Genrich, W. D. Hans, C. Jindrich Jr., *J. Med. Chem.* **2005**, *48*, 1256.
[7] J. M. Beaton, F. S. Spring, *J. Chem. Soc.* **1955**, 3126.
[8] M. Nose, M. Ito, K. Kamimura, M. Shimizu, Y. Ogihara, *Planta Med.* **1994**, *60*, 136.
[9] M. Noguchi, *Chem. Pharm. Bull*. **1978**, *26*, 2624.
[10] M. Noguchi, M. Kubo, T. Hayashi, M. Ono, *Chem. Pharm. Bull*. **1978**, *26*, 3652.
[11] M. Noguchi, Y. Hashimoto, *Shoyakugaku Zasshi* **1983**, *37*, 56.
[12] M. Noguchi, Y. Hashimoto, A. Kato, *Shoyakugaku Zasshi* **1985**, *39*, 101.
[13] M. Kamigauchi, N. Kambara, M. Sugiura, K. Iwasa, H. Ohishi, T. Ishida, *Helv. Chim. Acta* **2004**, *87*, 264.
[14] M. Kamigauchi, K. Kawanishi, H. Ohishi, T. Ishida, *Chem. Pharm. Bull.* **2007**, *55*, 729.
[15] M. L. Bender, M. Komiyama, 'Cyclodextrin Chemistry', Springer Verlag, **1978**.
[16] T. Steiner, G. Koellner, *J. Am. Chem. Soc.* **1994**, *116*, 5122.
[17] Program package Discovery Studio Modeling, Accelrys Software Inc., San Diego, CA, USA.
[18] F. A. Momany, R. Rone, *J. Comput. Chem*. **1992**, *13*, 888.
[19] Program package CHARMm of DS Modeling 1.1, Accelrys Software Inc., San Diego, CA, USA.
[20] 'Nanomaterial Cyclodextrin', The Society of Cyclodextrins, Japan, Yoneda Co. Ltd. Chiba, 2005, p. 235.
[21] B. Antigone, Y. Konstantina, P. Bruno, H. Eugene, *J. Org. Chem.* **1995**, *60*, 4017.
[22] H. J. Schneider, U. Buchheit, N. Becker, G. Schmidt, U. Siehl, *J. Am. Chem. Soc.* **1985**, *107*, 7027.

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