J Incl Phenom Macrocycl Chem (2009) 64:149–155
DOI 10.1007/s10847-009-9547-4

ORIGINAL ARTICLE

Binding behaviors of scutellarin with α-, β-, γ-cyclodextrins and their derivatives

Bo Yang · Li-Juan Yang · Jun Lin · Yong Chen · Yu Liu

Received: 27 November 2008 / Accepted: 30 January 2009 / Published online: 26 February 2009

Abstract A series of cyclodextrin/scutellarin inclusion complexes were prepared from α-cyclodextrin, β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin with scutellarin (SCU), and their inclusion complexation behaviors, such as stoichiometry, complex stability constants and inclusion mode, were investigated by means of UV/Vis spectroscopy, ^{1}H NMR and 2D NMR. The results showed that the SCU could be efficiently encapsulated in the cyclodextrin cavity in aqueous solution to produce complexes that were more soluble than free SCU. The enhanced binding ability of cyclodextrins towards SCU was discussed from the viewpoint of the size/shape-fit and multiple recognition mechanism between host and guest.

Keywords Scutellarin · Cyclodextrin · Inclusion complexation · Supramolecular chemistry

Introduction

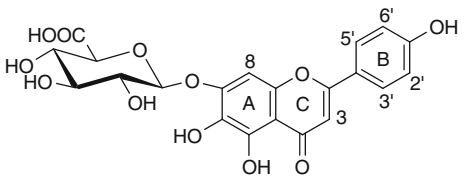
Breviscapine is the flavonoid constituents extracted from Chinese herb *Erigerin breviscapus* (Vant.) Hand.-Mazz., in which scutellarin (SCU, Scheme 1), a known flavone glycoside, is a primary active ingredient [1]. Breviscapine can significantly dilate blood vessels, improve microcirculation, increase cerebral blood flow and inhibit platelet aggregation activity. So, the preparation of breviscapine (injection breviscapine and breviscapine tablets) is extensively used in China for the treatment of cerebral infarction, cerebral thrombus, coronary heart disease and angina pectoris [2–4]. The latest research indicates that scutellarin have the neuroprotective effects and the anticoagulation effect. Moreover, scutellarin can also induce cell death in the human colon cancer cell line and protect against cerebral ischemia-reperfusion injury by many pathways of action [5–8].

However, it has been reported that the absolute bioavailability of scutellarin oral preparations was very low due to the poor solubility and hydrophobicity of scutellarin, and the short residence time of scutellarin in the circulation [9–11]. On the other hand, cyclodextrins (CDs, Scheme 2), a kinds of truncated-cone polysaccharides mainly made up of six to eight D-glucose monomers linked by α-1,4-glucose bonds with hydrophobic central cavity and hydrophilic outer surface, are known to be able to encapsulate model substrates to form host-guest complexes or superamolecular species as results to usually enhance drug solubility in aqueous solution and affect the chemical characterization of drugs [12–15].

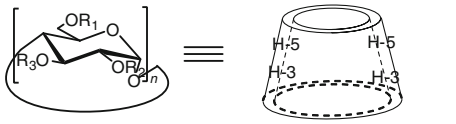
In order to improve the absolute bioavailability of scutellarin and prolong the duration of the drug in the circulation, effectively maintain the therapeutic drug levels in the blood for a long time, reduce the frequency of injection administration and therefore afford patient compliance, we investigated the interaction of scutellarin with a series of CDs such as αCD (α-Cyclodextrin), βCD (β-Cyclodextrin), γCD (γ-Cyclodextrin), HPβCD (2-hydroxypropyl-β-cyclodextrin) [M.S. (average molar degree of substitution) = 1.0] and TMβCD [heptakis(2,3,6-tri-O-methyl)-β- Cyclodextrin] in

B. Yang · L.-J. Yang · J. Lin (✉)
School of Chemistry, Key Laboratory of Medicinal Chemistry for Natural Resource (Ministry of Education), Yunnan University, 650091 Kunming, People's Republic of China
e-mail: linjun@ynu.edu.cn

B. Yang · Y. Chen · Y. Liu (✉)
Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, 300071 Tianjin, People's Republic of China
e-mail: yuliu@nankai.edu.cn; yuliu@public.tpt.tj.cn



Scheme 1 The structure of scutellarin



1 n=6 $R_1=R_2=R_3=H$ α–CD
2 n=7 $R_1=R_2=R_3=H$ β–CD
3 n=8 $R_1=R_2=R_3=H$ γ–CD
4 n=7 $R_1=R_2=R_3=CH_3$ TMβ–CD
5 n=7 $R_1=R_2=R_3=CH_2CH(CH_3)OH$ or H HPβ–CD

Scheme 2 The structure of cyclodextrin

aqueous solution. It is our special interest to explore the binding behaviors of native CDs and modified CDs with scutellarin, the solubilization effect of CDs toward scutellarin, which will provide a useful approach to achieve novel scutellarin formulation with high bioavailability.

Experimental section

Materials

Scutellarin was obtained by Kunming Pharmaceutical Industry Ltd. (PC > 98%) in Yunnan Province, P R China. αCD, rCD, βCD, TMβCD and HPβCD were commercially available.

Measurements

UV/Vis spectra were performed on a Shimadzu UV 3600 spectrophotometer, and pH 7.2 buffer solution was used in the spectral measurements. ^{1}H NMR experiments were performed on a Bruker Avance DRX500 spectrometer at 298 K in a deuterium oxide solution. Rotating-frame Overhauser effect spectroscopy (ROESY) experiments were run on a Bruker Avance DRX500 instrument. Samples were kept at least 24 h before measurement for thermal equilibration. All 2D NMR experiments were carried out in D_2O.

Preparation of βCD/SCU complex

SCU (0.03 mM, 13.9 mg) and βCD (0.01 mM, 12.6 mg) were completely dissolved in a mixed solution of ethanol and water (ca. 7 mL, v:v = 1:5), the mixture was stirred for 4 days at room temperature. After evaporating the ethanol from the reaction mixture, the uncomplexed SCU was removed by filtration. The filtrate was evaporated under the reduced pressure to remove the solvent and dried in vacuum to give βCD/SCU complex. ^{1}H NMR (500 MHz, D_2O, TMS): 3.60–3.90 (m, H-2–6 of βCD and some protons of SCU), 5.07 (s, H-1 of βCD), 6.46 (s, H-8 of SCU), 6.80 (s, H-3 of SCU), 6.84 (d, H-3′, 5′ of SCU), 7.65 (d, H-2′, 6′ of SCU).

Preparation of αCD/SCU complex

αCD/SCU complex was similarly prepared from αCD and SCU. ^{1}H NMR (500 MHz, D_2O, TMS): 3.55–3.80 (m, H-2–6 of αCD and some protons of SCU), 4.98 (s, H-1 of αCD), 6.08 (s, H-8 of SCU), 6.45 (s, H-3 of SCU), 6.57 (d, H-3′, 5′ of SCU), 7.27 (d, H-2′, 6′ of SCU).

Preparation of HPβCD/SCUcomplex

HPβCD/SCU complex was similarly prepared from HPβCD and SCU. ^{1}H NMR (500 MHz, D_2O, TMS): 1.09 (d, H-9 of HPβCD), 3.60–3.80 (m, H-2–8 of HPβCD and some protons of SCU), 5.08 (d, H-1 of HPβCD), 6.16 (s, H-8 of SCU), 6.50 (s, H-3 of SCU), 6.59 (d, H-3′, 5′ of SCU), 7.34 (d, H-2′, 6′ of SCU).

Results and discussion

Stoichiometry

The stoichiometry for the inclusion complexation of CDs with scutellarin was determined by Job's experiments. The Job's plots were determined from UV–vis data obtained in a pH 7.2 buffer. The total molar concentration (i.e., the combined concentration of scutellarin and CDs) was kept constant (5.0×10^{-5} M), but the molar fraction of scutellarin (i.e., [SCU]/([SCU] + [CD])) varied from 0.1 to 0.9. Figure 1 illustrates the Job's plot for the βCD/SCU system examined by UV spectra. In the concentration range, the plot for βCD showed a maximum at a molar fraction of 0.5, indicating the 1:1 inclusion complexation between host and guest. The same results were obtained in αCD and HPβCD with scutellarin.

Spectral titration

Quantitative investigation of the binding behavior of host CDs with scutellarin are examined in phosphate buffer solution by means of spectrophotometric titration method. From the absorbance intensity change induced by adding the host molecule, we can determine the complex stability

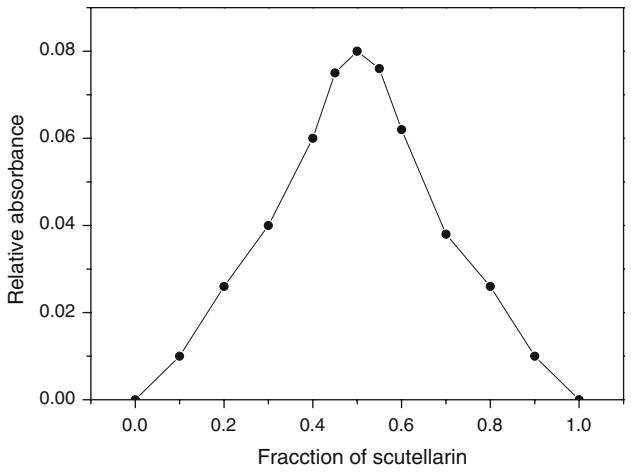


Fig. 1 Job's plot of the βCD/SCU system at 278 nm ([β-CD] + [SCU] = 5.0 × 10^{-5} M) in a pH 7.2 buffer

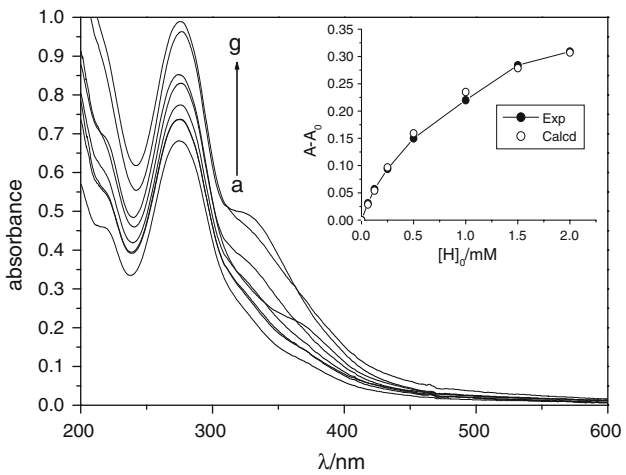


Fig. 2 Absorption spectral changes of scutellarin (3 × 10^{-5} M) upon addition of host βCD (0 ~ 1 × 10^{-3} M from *a* to *g*) in buffer solution (pH 7.2) and the nonlinear least squares analysis (*inset*) of the differential intensity (ΔA at 278 nm) to calculate the stability constant (K_S) and molar absortanse constant ($\Delta\varepsilon$)

constants (K_S). As the job's plot shows the 1:1 stoichiometry for the inclusion complexation of CDs with the guest molecule scutellarin, the inclusion complexation of guest (G) with host (H) is expressed by Eq. (1)

$$H + G \overset{K_S}{\rightleftharpoons} G \cdot H \tag{1}$$

The complex stability constants (K_S) were calculated for each host–guest combination from the nonlinear squares fit to Eq. (2)

$$\begin{aligned} K_s &= \frac{[CD][SCU]}{[CD \cdot SCU]} \\ &= \frac{\left([CD]_0 - \Delta A/\Delta\varepsilon\right)\left([SCU]_0 - \Delta A/\Delta\varepsilon\right)}{\Delta A/\Delta\varepsilon} \end{aligned} \tag{2}$$

where $[CD]_0$ and $[SCU]_0$ were initial concentrations of CDs and scutellarin respectively, equation (2) is achieved by equation (3):

$$\Delta A = \frac{\Delta\varepsilon\left([CD]_0 + [SCU]_0 + K_s\right) - \sqrt{(\Delta\varepsilon)^2\left([CD]_0 + [SCU]_0 + K_s\right)^2 - 4(\Delta\varepsilon)^2[CD]_0[SCU]_0}}{2} \tag{3}$$

where $[CD]_0$ and $[SCU]_0$ refer to the total concentrations of the guest and host and $\Delta\varepsilon$ is the proportionality coefficient, which may be taken as a sensitivity factor for the absorbance intensity change [16].

As illustrated in Figs. 2 and 3, the absorbance intensity of scutellarin gradually is increased with the stepwise addition of CDs. Using a nonlinear least squares curve-fitting method [17], we obtained the complex stability constant for each host–guest combination. Figures 2 and 3 illustrates the typical curve-fitting plots for the titrations of scutellarin at 278 nm with CDs, which shows the excellent fits between the experimental and calculated data. In the repeated measurements, the K_S values are reproducible within an error of ±5%. The K_S values obtained are listed in Table 1, along with the free energy changes of complex formation (ΔG_0) obtained upon addition of large excess of host.

Binding ability

For a substance to be included into the macrocycle, it is widely accepted that the first requirement is the size/shape fitting between host and guest molecules, and weak intermolecular forces such as ion–dipole, dipole–dipole, van der Waals, electrostatic, hydrogen bonding and hydrophobic interactions are known to cooperatively contribute to the inclusion complexation.

It is well known that each of α-CD, β-CD and γCD possesses a cyclic truncatedcone cavity with a height of 0.79 nm, but their average inner diameters are 0.50, 0.62 nm and 0.79 nm for α-CD, β-CD and and γCD, respectively. Therefore, the host–guest size matching may dominate the stability of the complexes formed between these CDs and scutellarin. From Table 1, we can see that

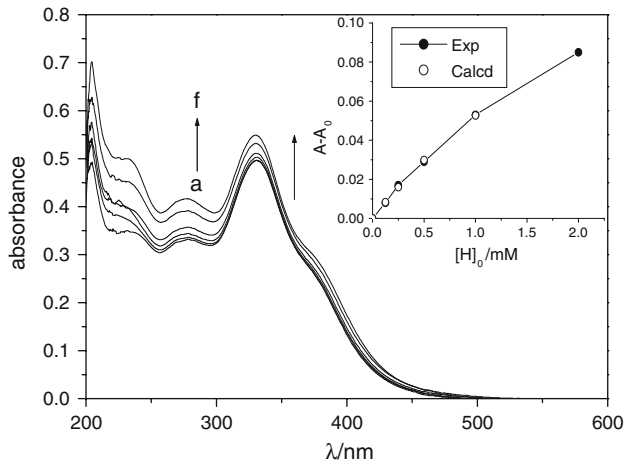


Fig. 3 Absorption spectral changes of scutellarin (3×10^{-5} M) upon addition of host βCD ($0 \sim 1 \times 10^{-3}$ M from *a* to *f*) in buffer solution (pH 10.5) and the nonlinear least squares analysis (*inset*) of the differential intensity (ΔA at 278 nm) to calculate the stability constant (K_S) and molar absortanse constant ($\Delta\varepsilon$)

Table 1 The stability constant ($\log K_S$) and Gibbs free energy change ($-\Delta G°$) for the host–guest complexes formed by scutellarin with cyclodextrins in aqueous buffer solution [(25.0 ± 0.1) °C]

Host	K_S	log K_S	$-\Delta G°$/kJ mol^{-1}	pH
αCD	210	2.32	13.24	7.20
βCD	634	2.80	15.98	7.20
βCD	510	2.70	15.44	8.20
βCD	420	2.62	14.96	9.20
βCD	317	2.50	14.26	10.5
HPβCD	1925	3.28	18.73	7.20
TMβCD	–[a]	–	–	7.20
γCD	–[a]	–	–	7.20

[a] The spectral changes of TMβCD and γCD with scutellarin are too small to calculate stability constant

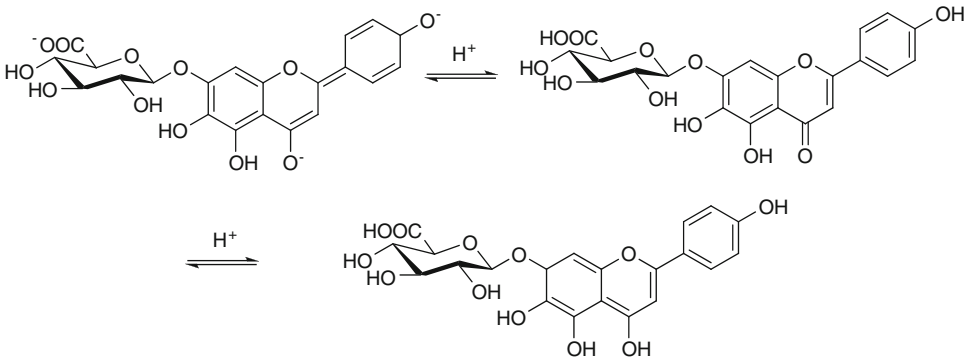
β-CD, which possessed a moderate cavity size, can better complex with the guest scutellarin, giving the stronger *K*s value than α-CD and γCD. In addition, it is demonstrated that the modified derivatives of CDs usually show the stronger binding ability toward model substrates than native CDs. Therefore, we also select TMβCD and HPβCD as host molecules to investigate their inclusion complexation abilities toward scutellarin. The results showed that the stability constant of SCU/HPβCD complex was 1925 M^{-1}. In contrast, the spectral changes of TMβCD with scutellarin are too small to calculate stability constant. The reason would be that, possessing a number of methoxyl groups instead of the hydroxyl groups at the exterior of CD cavity, the methylated βCD, such as TMβCD, had a smaller opening and a deeper cavity than native βCD [18, 19]. This structural feature of TMβCD would unfavor their inclusion complexation with SCU due to a relatively poor size-fit between host and guest. In addition, it is found that hydrogen bonding does contribute significantly to the stability of the inclusion complex [20]. As a result of complete methylation of the hydroxyl groups on cyclodextrins, the intermolecular hydrogen bonding between scutellarin and TMβCD is very weak or even negligible. Hence, the stability constant of TMβCD with scutellarin can not be obtained.

It was also interesting to compare the host–guest binding abilities at the different pH values. As can be seen in Table 1, the K_S of βCD/SCU complex decreases with pH. It is well known that drug/cyclodextrin complexation has been found to be better with the unionized drug than with the ionized one due to the dipole-electrostatic interactions [21]. The ionization degree of scutellarin increases with pH (Scheme 3). As a result, βCD forms a relatively strong complex with the unionized scutellarin.

Inclusion mode

In order to explore the possible inclusion mode of CD/SCU complexes, we compared the ^{1}H NMR spectra of SCU in the presence of host CDs (Fig. 3), where the ^{1}H resonances of αCD, βCD and HPβCD were assigned according to the reported method [22, 23]. As illustrated in Fig. 4, a majority of SCU (6H) display the chemical shifts at δ 6.0–8 ppm, which are distinct from the CD protons. As can be seen from Table 2, after inclusion complexation with SCU, the H-3 proton of HPβCD shifted 0.024 ppm and that of βCD shifted 0.022 ppm, and the H-5 proton of HPβCD

Scheme 3 Ionized and unionized conformation of scutellarin in solution



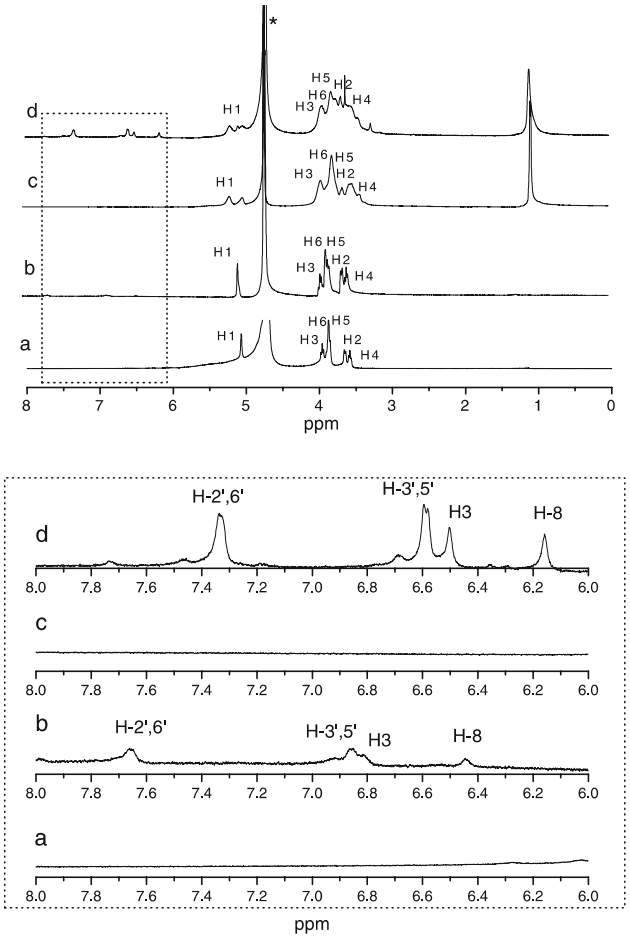


Fig. 4 1H NMR spectra of *β*CD and HP*β*CD in the absence and presence of SCU in D2O at 25 °C, respectively. **a** *β*CD, (**b**) *β*CD/SCU complex, (**c**) HP*β*CD, (**d**) HP*β*CD/SCU complex (*asterisk highlights the water peak, the window shows the enlarged NMR spectrum from 6 to about 8 ppm*)

shifted 0.060 ppm and that of *β*CD shifted 0.017 ppm. Because both H-3 and H-5 protons are located in the interior of CD cavity, and H-3 protons are near the wide side of cavity while H-5 protons near the narrow side, this phenomenon may indicate that SCU should be included in the *β*CD and HP*β*CD cavity. In contrast, a weak shift is observed on the *δ* values of H-3 and H-5 protons of αCD. However, the protons of SCU were still at *δ* 6.0–8 ppm, indicating that αCD only weakly associate with scutellarin to form the inclusion complex.

Two-dimensional (2D) NMR spectroscopy has recently become an important method to obtain information about the spatial proximity between the atoms of host and guest by observing the intermolecular dipolar cross-correlations. Two protons, which are closely located in space, can produce a NOE cross-correlation between the relevant protons in NOESY or ROESY spectrum. The presence of NOE cross-peaks between protons from two species indicates spatial contacts within 0.4 nm [24]. To gain more conformational information, we used 2D ROESY to study the inclusion complexes. Fig. 5 (inset) shows a partial contour plot of 2D-ROESY spectra of the inclusion complex of scutellarin with HP*β*CD. ROESY spectrum of the SCU/HP*β*CD complex shows appreciable correlation between H-3', 5' protons of the C-ring of scutellarin with H-5 and H-3 protons of the cyclodextrin, and no correlation between H-2', 6' protons of the scutellarin with H-5 or H-3 protons of the cyclodextrin, indicating that the entire phenol is included in the HP*β*CD cavity and B-ring partly protrudes towards the primary hydroxyl group. It is fairly noteworthy that, there are two intermolecular cross-peaks, the first one between H-3 protons of scutellarin with H-3 protons of HP*β*CD, the second one between H-8 protons of the A-ring with H-3 of HP*β*CD indicating that scutellarin inserted in the cyclodextrin cavity with the A-ring and C-ring orienting towards the secondary hydroxyl group. Along with the result of the 1:1 inclusion stoichiometry observed in the Job's plot, the possible inclusion mode of HP*β*CD/scutellarin complex was illustrated in Fig. 6.

Table 2 The chemical shifts (*δ*) of *β*CD, HP*β*CD, α-CD, *β*CD/SCU complexes, HP*β*CD/SCU complexes and α-CD/SCU complexes in D_2O at 25 °C

		δ (ppm)					
		*β*CD	*β*CD complex	HP*β*CD	HP*β*CD complex	α-CD	α-CD complex
H-1 of CD	d	5.068	5.062	5.085	5.078	4.979	4.979
H-2 of CD	dd	3.650	3.646	3.636	3.654	3.569	3.572
H-3 of CD	dd	3.961	3.939	3.928	3.904	3.905	3.907
H-4 of CD	dd	3.582	3.580	3.503	3.595	3.508	3.511
H-5 of CD	m	3.854	3.837	3.648	3.728	3.775	3.779
H-6 of CD	dd	3.873	3.868	3.778	3.791	3.795	3.797
H-2',6'of SCU	d	–	7.649	–	7.336	–	7.271
H-3',5'of SCU	d	–	6.853	–	6.588	–	6.565
H-3 of SCU	s	–	6.802	–	6.502	–	6.448
H-8 of SCU	s	–	6.455	–	6.158	–	6.080

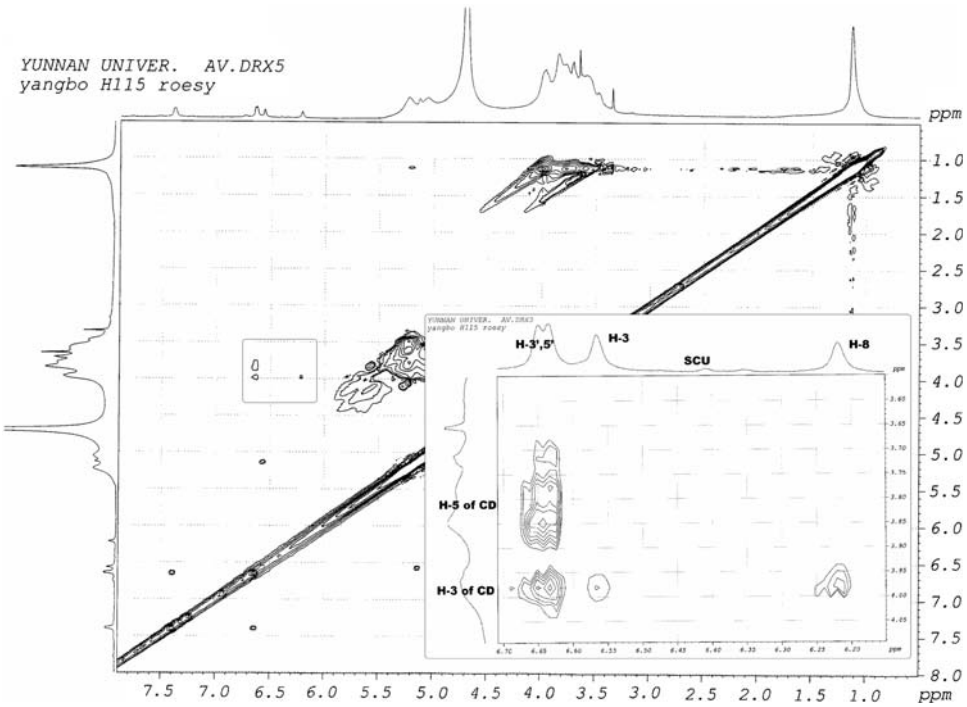


Fig. 5 ROESY spectrum of HPβCD/SCU complex in a D_2O

Water solubility

The water solubility of CD–scutellarin complex is assessed by preparation of its saturated solution [25]. An excess amount of complex was put into 5 mL of water (pH ca. 7), and the mixture was stirred for 1 h. After removing the insoluble substance by filtration, the filtrate is evaporated under reduced pressure to dryness and the residue is dosed by weighing method. The results show that the water solubility of βCD/scutellarin, HPβCD/scutellarin and αCD/scutellarin complexes comparing with that of scutellarin (ca. 160 μg/mL), is dramatically increased to approximately 9.0 and 10.3 mg/mL (calculated as scutellarin residue), respectively. In the control experiment, a clear solution is obtained after dissolving βCD/scutellarin (23.9 mg), HPβCD/scutellarin (32.7 mg) or αCD/scutellarin (20.9 mg) complex, respectively, which is equivalent to 9.0, 10.3 and 8.2 mg of scutellarin, in 1 mL of water at room temperature. This subsequently confirms the reliability of the obtained satisfactory water solubility of CD/scutellarin complexes, which will be beneficial to the utilization of this compound as medicine products.

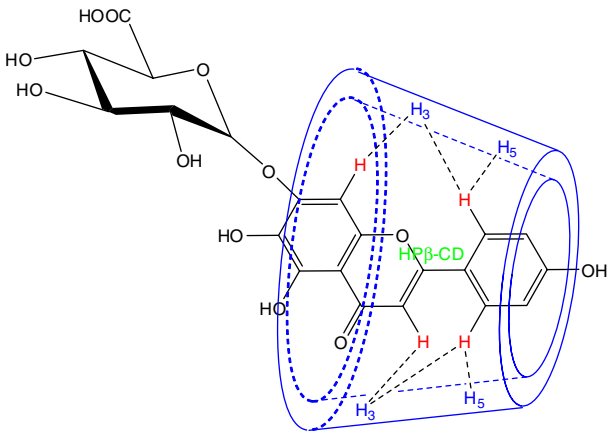


Fig. 6 Possible inclusion mode of HPβCD/SCU complexe

Conclusion

In summary, the binding behaviors of several cyclodextrins with scutellarin were investigated. The results showed that CDs could enhance the water-solubilities of scutellarin. Considering the shortage of application of scutellarin, these complexes should be regarded as an important choice in the design of novel formulation of scutellarin for herbal medicine.

Acknowledgments This work was supported by the Opening Foundation of State Key Laboratory of Elemento-Organic Chemistry of Nankai University (0607 and 0704), which is gratefully acknowledged.

References

1. Zhang, W.D., Chen, W.S., Wang, Y.H., Yang, G.J., Kong, D.Y., Li, H.T.: Studies on the flavone glycosides from the extract of Erigeron breviscapus. Chin. Traditional Herb. Drugs. **31**, 565–568 (2000)

2. Zhang, J., Li, X.S., Zhang, W.D.: Recent progress on pharmacologic activity and chemical components of breviscapine. J. Pharm. Pract. **20**, 103–107 (2002)
3. Deng, T.F.: Clinical application of breviscapine injection in Chinese patients. China Pharm. **11**, 728–730 (2002)
4. Si, S.L., Xu, L.Y.: Clinical application of breviscapine formulations. Chin. J. Clin. Pharm. **13**, 408–410 (2004)
5. Ju, W.Z., Chun, J.H., Tan, R.X., Xiong, N.N.: Study on metabolites of scutellarin in gastrointestinal tract by UPLC-MS/MS method. Chin. J. Clin. Pharmacol. Ther. **11**, 292–295 (2006)
6. Jiang, X.H., Li, S.H., Lan, K., Yang, J.Y., Zhou, J.: Study on the pharmacokinetics of scutellarin in dogs. Acta. Pharm. Sinica. **38**, 371–373 (2003)
7. Liu, Y.M., Lin, A.H., Chen, H., Zeng, F.D.: Study on pharmacokinetics of scutellarin in rabbits. Acta. Pharm. Sinica. **38**, 775–778 (2003)
8. Ge, Q.-H., Zhou, Z., Zhi, X.J., Ma, L.L.: Pharmacokinetics and absolute bioavailability of breviscapine in Beagle dogs. Chin. J. Pharm. **34**, 618–620 (2003)
9. Hong, H., Liu, G.Q.: Protection against hydrogen peroxide-induced cytotoxicity in PC12 cells by scutellarin. Life Sci. **74**, 2959–2973 (2004)
10. David, G., Yian, H.L., Eng, S.O.: Inhibitory effects of a chemically standardized extract from scutellaria barbata in human colon cancer cell lines, LoVo. J. Agric. Food Chem. **53**, 8197–8204 (2005)
11. Shuai, J., Dong, W.W.: Experimental research of PKC inhibitor. Erigeron breviscapus on the ischemic/reperfusional brain injury. Chin. Pharmacol. Bull. **14**, 75–77 (1998)
12. Szejtli, J.: Cyclodextrin Technology, pp. 450–455. Kluwer Academic Publisher, Dordrecht (1988)
13. Uekama, K., Hirayama, F., Irie, T.: Cyclodextrin drug carrier systems. Chem. Rev. **98**, 2045–2076 (1998)
14. Loftsson, T., Järvinen, T.: Cyclodextrins in ophthalmic drug delivery. Adv. Drug Deliv. Rev. **36**, 59–79 (1999)
15. Loftsson, T., Brewster, M.E.: Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci. **85**, 1017–1025 (1996)
16. Inoue, Y., Yamamoto, K., Wada, T., Everitt, S., Gao, X.M., Hou, Z.J., Tong, L.H., Jiang, S.K., Wu, H.M.: Inclusion complexation of (cyclo)alkanes and (cyclo)alkanols with 6-O-modified cyclodextrins. J. Chem. Soc. Perkin Trans. 2, 1807–1816 (1998)
17. Liu, Y., Li, B., Wada, T., Inoue, Y.: Novel O-Phenylenediseleno bridged Bis(β-cyclodextrin)s complexes with Platinum(IV) and Palladium(II) Ions. Supramol. Chem. **10**, 279–285 (1999)
18. Reinhardt, R., Richter, M., Mager, P.P.: Investigation of the conformational behaviour of permethylated cyclodextrins by molecular modelling. Carbohydrate Res. **291**, 1–9 (1996)
19. Kano, K., Nishiyabu, R., Asada, T., Kuroda, Y.: Static and dynamic behavior of 2:1 inclusion complexes of cyclodextrins and charged porphyrins in aqueous organic media. J. Am. Chem. Soc. **124**, 9937–9944 (2002)
20. Yi, Z.-P., Chen, H.-L., Huang, Z.-Z., Huang, Q., Yu, J.-S.: Contributions of weak interactions to the inclusion complexation of 3-hydroxynaphthalene-2-carboxylic acid and its analogues with cyclodextrins. J. Chem. Soc. Perkin Trans. 2, 121–127 (2000)
21. Linares, M., de Bertorello, M.M., Longhi, M.: Solubilization of naphthoquinones by complexation with hydroxypropyl-β-cyclodextrin. Int. J. Pharm. **159**, 13–18 (1997)
22. Liu, Y., Chen, C.-S., Chen, Y., Lin, J.: Inclusion complexes of azadirachtin with native and methylated cyclodextrins: solubilization and binding ability. Bioorg. Med. Chem. **13**, 4037–4042 (2005)
23. de Araújo, M.V.G.: Sulfadiazine/hydroxypropyl-β-cyclodextrin host–guest system: characterization, phase-solubility and molecular modeling. Bioorg. Med. Chem. **16**, 5788–5794 (2008)
24. Correia, I., Bezzenine, N., Ronzani, N., Platzer, N., Beloeil, J.-C., Doan, B.-T.: Study of inclusion complexes of acridine with β- and (2,6-di-O-methyl)-β-cyclodextrin by use of solubility diagrams and NMR spectroscopy. J. Phys. Org. Chem. **15**, 647–659 (2002)
25. Montassier, P., Duchêne, D., Poelman, M.C.: Inclusion complexes of tretinoin with cyclodextrins. Int. J. Pharm. **153**, 199–209 (1997)