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Host-Guest Sensory System of Sodium Anthranilate-Modified β-Cyclodextrin: Molecular Recognition Properties

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Abstract. Sodium anthranilate-modified β-cyclodextrin (**1**) has been prepared as a sensor for detecting organic compounds including terpenoids and steroids. **1** shows a pure monomer fluorescence whose intensity is increased or decreased upon addition of the guest species examined. In this system, the sodium anthranilate moiety acts either as a spacer, which enables the cyclodextrin to form a 1 : 1 guest complex by narrowing the β-cyclodextrin cavity or acts as a hydrophobic cap. **1** shows a higher sensitivity for terpenoids than for steroids, but has a higher selective molecular recognition ablity for steroids than for terpenoids.

Key words: Modified cyclodextrin, fluorescent sensory system, steroids, terpenoids.

1. Introduction

Cyclodextrins, which are torus-shaped cyclic oligomers of D-glucopyranose and named α-, β-, and γ- for the hexa, hepta, and octamers, respectively, can include organic compounds in their cavities in aqueous solution [1]. Cyclodextrins are basically inert with respect to optical spectroscopy, because they have no chromophores in their structures. Cyclodextrins can, however, become spectroscopically active compounds by modification with chromophores, and spectroscopically inert guests can probably be recognized by the spectral change of modified cyclodextrins upon guest addition. We have reported the synthesis of modified-cyclodextrins, which show unique host-guest binding behavior in aqueous solution [2]. Recently, we reported the sensory system of dansylglycine-modified cyclodextrins for steroids,

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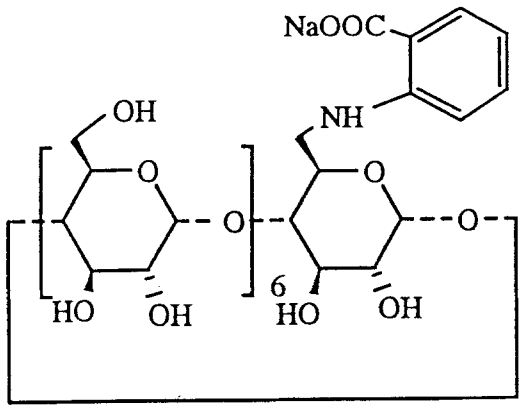

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Scheme 1.

in which the fluorescent intensity is changed upon addition of a guest molecule [3]. For further extension of the work, we prepared a new cyclodextrin derivative (**1**) modified with sodium anthranilate, which is a fluorescent compound, on the rim of β-cyclodextrin. **1** shows a unique host-guest complexation pattern, which is of a different type from that reported previously.

Here we would like to report the sensory ability of **1** for steroids and terpenes in aqueous solution.

2. Experiment

2.1. Preparation of Sodium Anthranilate – Modified β-Cyclodextrin (1)

2.1.1. *Sodium Anthranilate*

5 g (36.50 mM) of anthranilic acid was added to a solution of 1.81 g (45.25 mM) of sodium hydroxide in 18 mL of water. The reaction mixture was stirred for 4 h at room temperature and evaporated in vacuo. The precipitates were filtered and recrystallized from EtOH to give a pure compound (4.86 g, 84%).

mp 290 °C (decompose) IR (KBr) 1550 cm^{-1}, 1350 cm^{-1} .

2.1.2. *6-Iodo-6-Deoxy-β-Cyclodextrin*

A mixture of 7 g (5.19 mmol) of 6-*O*-*p*-toluenesulfonyl-β-cyclodextrin and 12.9 g (77.7 mM) of potassium iodide in 126 mL of *N*, *N'*-dimethylformamide (DMF) was heated for 7 h at 80 °C. After cooling, the reaction mixture was poured into 1000 mL of acetone. The precipitates were collected by filtration and dried. Recrystallizaton of the residue from the mixed solvent of 1-BuOH,EtOH and water (5 : 4 : 3 by volume) yielded 5 g of 6-iodo-6-deoxy-β-cyclodextrin (71.3%).

R_f 0.33 (l-butanol : ethanol : water 5 : 4 : 3 by volume). Found: C, 37.47; H, 6 09%. Calcd. for $C_{42}H_{69}IO_{34}\cdot 6H_2O$: C, 37.28; H, 6.03%.

2.1.3. 6-*Deoxy*-6-(2-*Sodium Benzene Carboxylate*-1-*Amino*)-β-*Cyclodextrin* (**1**)

1 g (0.80 mM) of 6-iodo-6-deoxy-β-cyclodextrin was added to a solution of 0.144 g (0.91 mM) of sodium anthranilate in 30 ml of DMF. The reaction mixture was heated at 80 °C for 3 h under a nitrogen atmosphere. After cooling, the reaction mixture was poured into 300 mL of acetone. The resultant precipitates were filtered and dried. The crude product was purified with a Sephadex G-15 column (3 × 90 cm) to afford 0.35 g (31% isolated yield) of pure **1**. R_f 0.41 (l-butanol, ethanol, water 5 : 4 : 3 by volume). ^{1}H-NMR (DMSO-d_6) = 3.3–3.6 (44H, m, C_2-C_4H of cyclodextrin), 4.65 (6H, m, O_6H), 4.85 (7H, s, C_1H), 5.74 (14H, br.s, O_2H, O_3H), 6.50 (1H, t, J = 9Hz, aromatic-H), 6.60 (1H, s, -NH), 6.76 (1H, d, J = 9Hz, aromatic-H), 7.23 (1H, t, J = 9Hz, aromatic-H), 7.70 (1H, d, J = 9Hz, aromatic-H). $C_{49}H_{74}O_{36}NNa{\cdot}6H_2O$. Calcd. for C, 42.52; H, 6.26; N, 1.01. Found C, 42.79; H, 6.38; N, 0.95. MS(FAB): 1276 ([M+H$^+$]).

2.2. Measurement

Fluorescence spectra were measured at 25 °C with a Hitachi F-3010 spectrofluophotometer. The excitation wavelength was 330 nm or 250 nm. Excitation and emission slits were 5 nm. 5 μL of guest species dissolved in dimethyl sulfoxide (DMSO) or methanol (0.5 and 0.05 M) were injected into a solution of **1** in aqueous solution (2.5 mL) to make sample solutions with a host concentration of 1×10^{-6} M and guest concentrations of 0.1 and 1.0 mM.

3. Results and Discussion

In the last decade, we have reported the synthesis and host-guest complexation behaviors of spectroscopically active cyclodextrins that contain chromophores such as naphthalene, anthracene, and pyrene [2]. These cyclodextrin derivatives show remarkable variations in their circular dichroism, absorption, and fluorescence spectra associated with the formation of inclusion complexes.

It is recognized that some modified cyclodextrins can act as a sensor to detect organic compounds because they have the selectivity and sensitivity of recognition ability towards guest molecules [3, 4]. In previous papers, we reported the sensory systems of β- and γ-cyclodextrin with a dansylglycine moiety, which show the enhancement or decrement of fluorescence intensity upon guest addition [3]. For the β-cyclodextrin derivative, the appended moiety acts as a hydrophobic cap, in which the moiety is excluded from the cyclodextrin cavity upon guest addition. On the other hand, for the γ-cyclodextrin derivative, the dansyl glycine moiety acts in two ways, viz., as a spacer, in which the dansyl glycine moiety is included to narrow the large γ-cyclodextrin cavity (Figure 1) or as a hydrophobic cap (Figure 2). Figure 3 shows fluorescence spectra of **1** in aqueous solution in the presence and

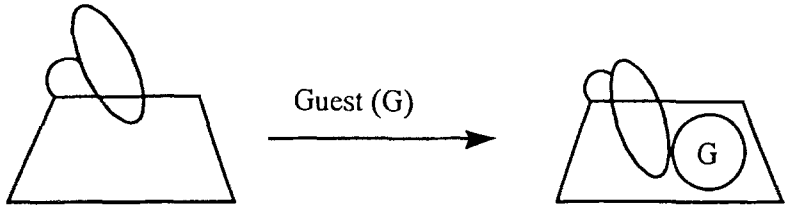


Fig. 1. Induced-fit type of space regulation by the appended moiety for inclusion of a guest molecule (**G**) in the cyclodextrin cavity.

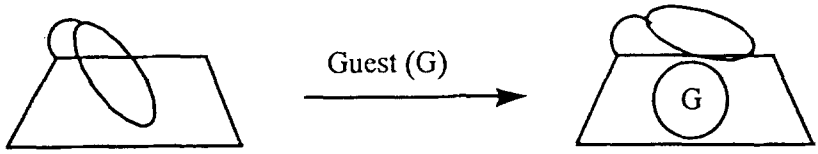


Fig. 2. Induced-fit type complexation of modified cyclodextrin with a pendant moiety acting as a hydrophobic cap for inclusion of a guest molecule (**G**) in the cyclodextrin cavity.

absence of l-borneol. The spectrum of **1**, alone, exhibits a fluorescence peak at 418 nm, and the fluorescence intensity increases with increasing l-borneol concentration. This guest-induced fluorescence enhancement has never been observed before for danyl-modified β-cyclodextrin and the other modified β-cyclodextrins reported by us. However, this fluorescence enhancement has been shown by naphthalene-appended γ-cyclodextrin [5] and dansyl-modified γ-cyclodextrin. To further confirm the movement of the appended-moiety in the cavity of the cyclodextrin, the fluorescence spectra of **1** were recorded in the mixed solvent of water and DMSO, in which the DMSO content increased from 0 to 80 vol.-%. It is well known that the inclusion ability of cyclodextrin is the highest in pure water and decreases in a less polar solvent such as DMSO [6]. To avoid the influence of the DMSO on the fluorescence spectra, the excitation wavelength was changed from 330 nm to 250 nm.

Figure 4 shows the fluorescence intensity of **1** at 418 nm in various aqueous DMSO solutions. The fluorescence intensity increases as the DMSO content is increased from 0 to 20 vol.-%, and then decreases with increasing DMSO content; the intensity of the fluorescence spectra decreases from a maximum value of 50 to 5 when 80 vol.-% DMSO aqueous solution was used. This suggests that the appended moiety is excluded from the cavity of cyclodextrin. As the solvent is changed from 0 to 20 vol.-% of DMSO aqueous solution, the sodium anthranilate moiety acts as a spacer that enables the cyclodextrin to form a 1 : 1 DMSO complex by narrowing the large β-cyclodextrin cavity. The values of the fluorescence intensity of **1** in pure water and 20 vol.-% DMSO aqueous solution, alone and including l-borneol at 1.0 mM (M = mol dm^{-3}) were obtained. The values of the fluorescence intensity of **1** in

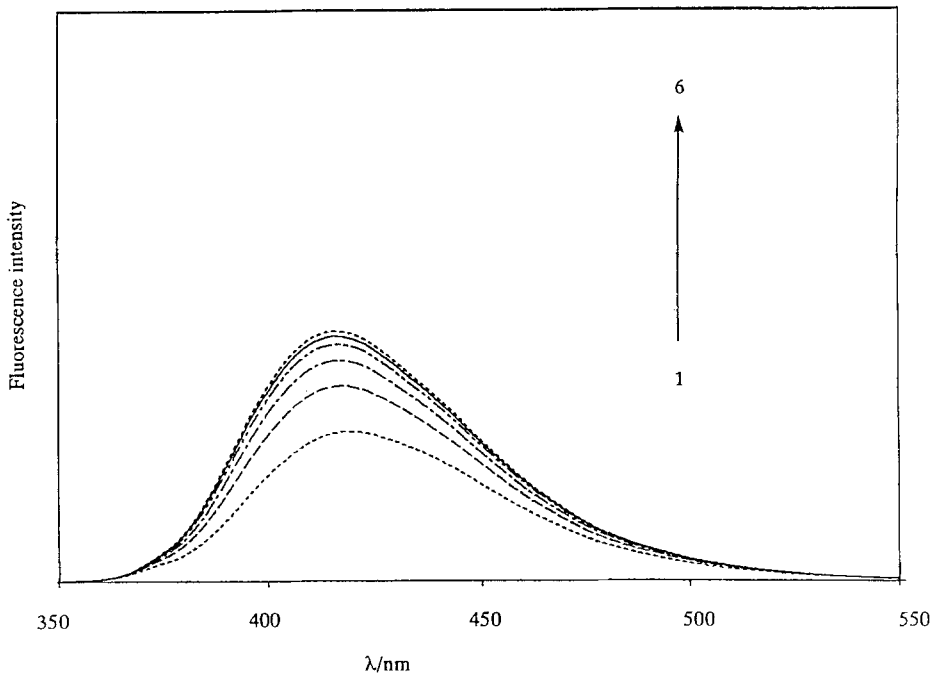
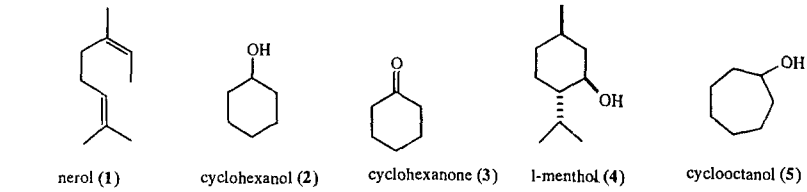


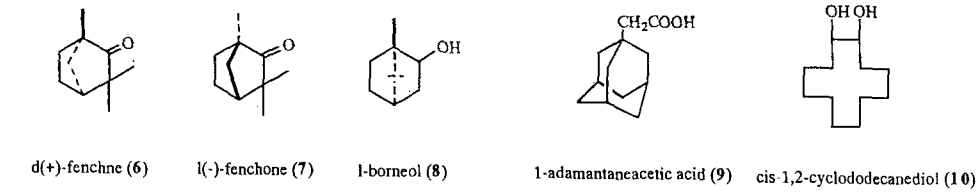
Fig. 3. Fluorescence spectra of **1** (1×10^{-6} M) in aqueous solution at various concentrations of l-borneol (1, 0; 2, 4.0×10^{-6}; 3, 1.20×10^{-5}; 4, 2.0×10^{-5}; 5, 3.19×10^{-5}; 6, 4.78×10^{-5} M). Excitation wavelength was 330 nm.

20 vol.-% DMSO aqueous solution is increased from 46.6 to 55.7 upon addition of l-borneol. However, the intensity is dramatically increased in pure water, from 27.7 to 54.8 on addition of l-borneol, which are almost the same values for each other. This fact means that the appended moiety is included in the cyclodextrin cavity to almost the same extent. To calculate the sensory ability of modified cyclodextrins in pure water, we used the $\Delta I/I^0$ value as reported previously [3]. Here, ΔI is I^0-I, where I^0 is the fluorescence intensity for the host, alone, and I is for a mixture of host and guest.

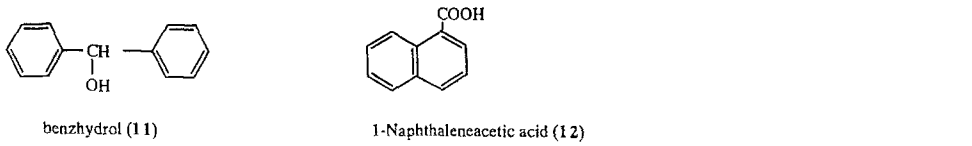
Figure 5a shows the $\Delta I/I^0$ values of **1** obtained with 12 guests at 1.0 mM (M = mol dm^{-3}), except for 1-adamantaneacetic acid (**9**) and cis-1,2-cyclododecanediol (**10**), which were examined at 0.1 mM because 1.0 mM of **9** and **10** are not soluble in pure water. The $\Delta I/I^0$ values range from −0.087 to −0.865. This suggests that the complexation behavior of **1** with a guest molecule was affected by the molecular structure and size. Compound **1** recognizes seven-membered and bi-cyclic ring molecules, such as **5**, **6**, **7** and **8** with the highest sensitivity and six-membered ring molecules, such as **2** and **3** with the next highest sensitivity. **9** and **10**, which are tricyclic or twelve-membered ring molecules, are detected with remarkably high sensitivities, even at one tenth concentration. On the other hand, aromatic



nerol (1) cyclohexanol (2) cyclohexanone (3) l-menthol (4) cyclooctanol (5)

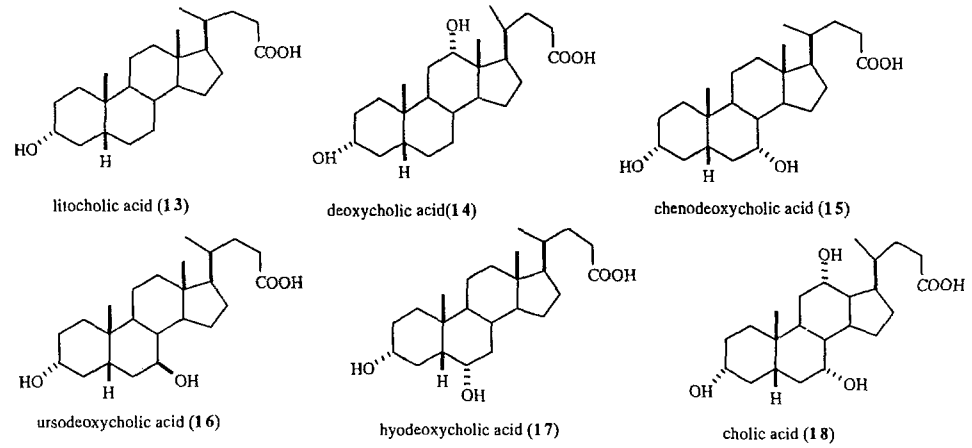


d(+)-fenchne (6) l(-)-fenchone (7) l-borneol (8) 1-adamantaneacetic acid (9) cis-1,2-cyclododecanediol (10)



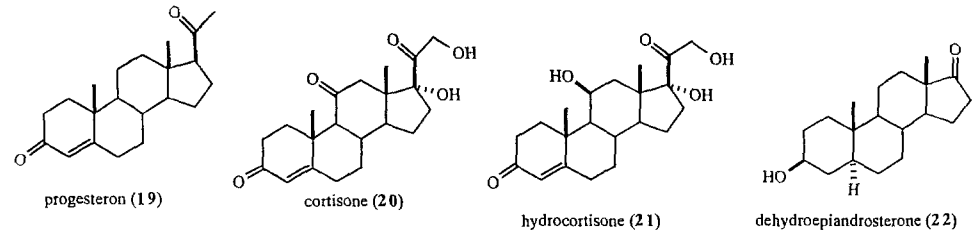
benzhydrol (11) 1-Naphthaleneacetic acid (12)

Scheme 2. Acryclic, cyclic and aromatic guest molecules.



litocholic acid (13) deoxycholic acid(14) chenodeoxycholic acid (15)

ursodeoxycholic acid (16) hyodeoxycholic acid (17) cholic acid (18)



progesteron (19) cortisone (20) hydrocortisone (21) dehydroepiandrosterone (22)

Scheme 3. Steroidal guest molecules.

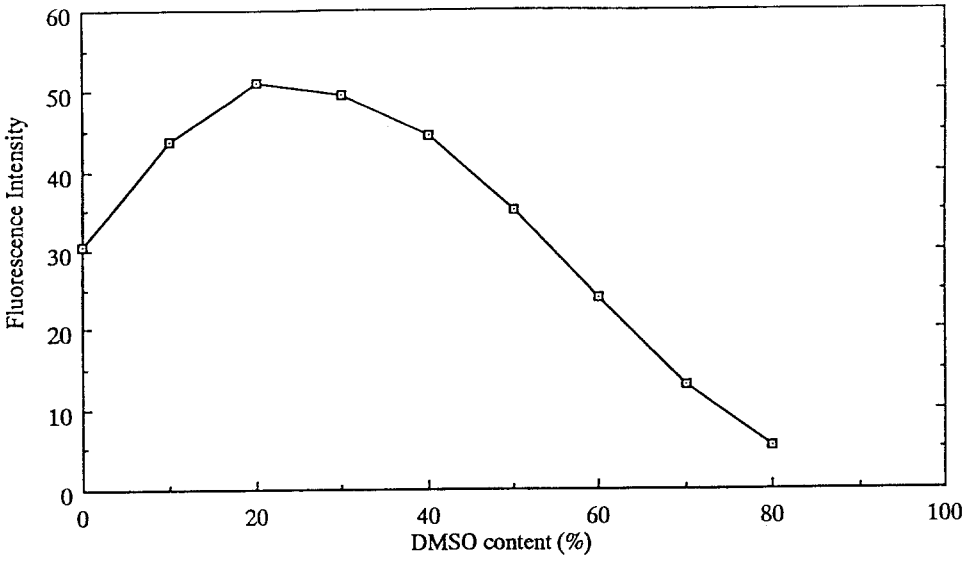


Fig. 4. The fluorescence intensity at 418 nm of **1** (1×10^{-6} M) in various DMSO aqueous solutions. Excitation wavelength was 250 nm.

derivatives or non-cyclic guest molecules, such as **1**, **11** and **12** are only weakly detected by this system. Steroids are biologically important substances, and it seems interesting to investigate how they are detected by **1**. Figure 5b shows the $\Delta I/I^0$ values of **1** obtained with steroids at 0.1 mM rather than at 1.0 mM as the steroids are not sufficiently soluble in pure water. Among the steroidal molecules, the $\Delta I/I^0$ values for compounds **16**, **19**, **20** and **21** are positive, which means the fluorescence spectra decrease on guest molecule addition. Ueno *et al.* reported the sensory system of pyrene-appended γ-cyclodextrin, in which some steroids act as a quencher to decrease the intensity of fluorescence and caused an anomaly in the fluorescence intensity of pyrene-modified γ-cyclodextrin (**23**) [4]. As with **23**, those guests probably act as quenchers in the system of **1**. It is obvious that chenodeoxycholic acid (**15**) and hyodeoxycholic acid (**17**) are detected with remarkably high sensitivities, exhibiting values of -0.23 and -0.33 for $\Delta I/I^0$, respectively. It is interesting that ursodeoxycholic acid (**16**) is detected with a positive value for $\Delta I/I^0$, even though **16** has no α,β-unsaturated moiety in the structure. Chenodeoxycholic acid (**15**) and ursodeoxycholic acid (**16**) are different only in the configuration of the hydroxy group at C-7 of the steroidal framework. However, the value and sign of those guests of **1** for $\Delta I/I^0$ are totally different. This fact suggests that stereochemical inversion of the hydroxy group seems to vary the effect of the sensory ability of this system. Deoxycholic acid (**14**), which bears an extra hydroxy group compared with lithocholic acid (**13**), was detected with lower sensitivity. Cholic acid (**18**), which bears an additional hydroxy group compared

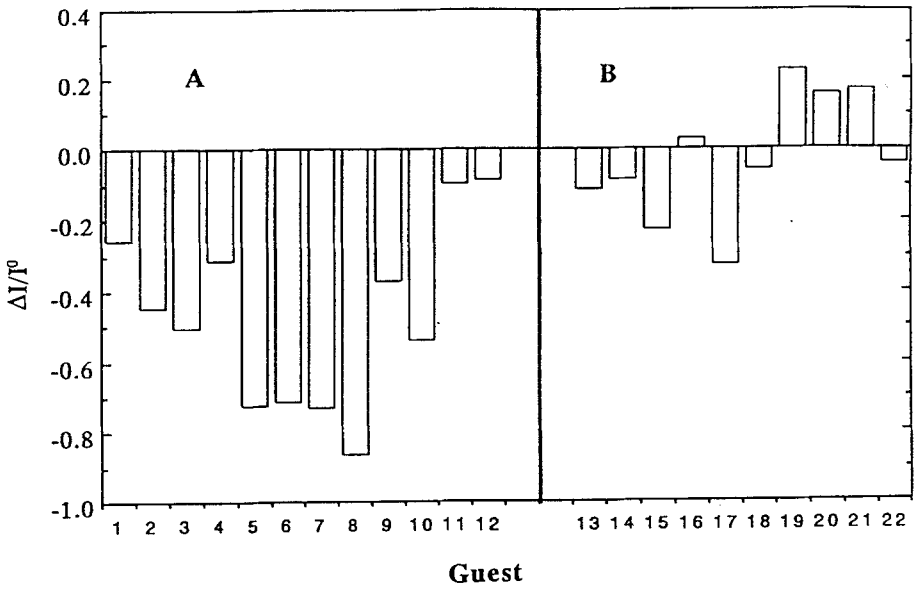


Fig. 5. The sensitivity factor $\Delta I/I^0$ of **1** (1×10^{-6} M) for various guests.

to **15**, was hardly detected, probably due to its increased polarity. When 0.1 mM of l-borneol was used, the value of $\Delta I/I^0$ is -0.468 suggesting that **1** shows a higher sensitivity for terpenoids than steroids.

4. Conclusion

The sensory system of **1** exhibits high sensitivity for including small guest molecules and steroidal compounds. It is significant that steroidal compounds, which are biologically significant substances, were detected by this system with high sensitivities at very low concentration. It is the first example, to our knowledge, that compound (**1**) shows a different host-guest complexation pattern. Further work with many other guests is needed to clarify the relationships between guest and sensitivity of this system. We are attempting to prepare the di-sodium anthranilate appended γ-cyclodextrin derivative to construct a sensor system with higher sensitivity.

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