Supramolecular Encapsulation of Pulegone from Oriental Herb, Schizonepeta tenuifolia Briquet by β- and γ-Cyclodextrins

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Pulegone is a naturally occurring monoterpane ketone obtained from the essential oils of a variety of plants such as Nepeta cataria (catnip), Mentha piperita, and pennyroyal. It was reported that the content of pulegone in Schizonepeta tenuifolia Briquet, the dried aerial part of Jingjie, is more than 0.5% and as high as 4.1% in its spike. Pulegone has bio-functional activities including antinociceptive, antibacterial, fumigant and acaricidal activity. As pulegone was more, the negligible cytotoxic effects and the ability to permeate biological membranes of cyclodextrins afford important advantages in applications such as food and flavors, pharmaceuticals, cosmetics, environment protection, bioconversion, packing and the textile industry.

For the purpose of further biochemical applications, we have investigated supramolecular interactions between pulegone molecules and water-soluble host molecules such as β- and γ-cyclodextrins to solubilize pulegone in water. The cavities of β- and γ-cyclodextrins are large enough for pulegone to accommodate in the cavity while that of α-cyclodextrin is too small. In addition, β-cyclodextrin is the most readily accessible, the lowest-priced and generally the most useful among cyclodextrin homologues. We here report the investigation on the supramolecular encapsulation of pulegone by β- and γ-cyclodextrins.

We extracted and purified pulegone from Schizonepeta tenuifolia Briquet for the future studies on phytochemicals. The purity of yielded pulegone exceeds over 99%, which was estimated by the 1H NMR spectrometry, gas chromatography (GC), and GC-Mass techniques. Scheme 1 illustrates the inclusion of pulegone in the cavity of cyclodextrins (CDs) with their chemical structures. The encapsulation of pulegone by CDs was performed by direct mixing pulegone with aqueous solution of CDs at room temperature. The inclusion complexes 1 (pulegone@β-CD) and 2 (pulegone@γ-CD) were isolated after simple work-up processes. The inclusion complexes were characterized by various spectroscopic methods as well as elemental and thermogravimetric analyses.

The formation of supramolecular complex between pulegone and CDs was supported by IR spectroscopy which is a useful tool to prove the presence of both host and guest components in an inclusion complex. The IR spectra of the inclusion complexes (1 and 2), CDs, and pulegone are shown in Figure 1. All changes of the stretching frequency for pulegone can be not clearly observed upon supramolecular complexation with β or γ-CDs because large parts of the characteristic bands of pulegone were overlapped with those of β or γ-CDs. But we could evidently observed the C=O stretching vibration band originated from the complexed pulegone at 1678 and 1687 cm⁻¹ for 1 and 2, respectively. The intensities of the bands were noticeably reduced.
owing to the complexation. An additional key observation to support the inclusion of pulegone in CDs is the sharpening with the decreased intensity of the peak between 3500 and 3000 cm$^{-1}$ in the spectrum of the inclusion complexes. The broad bands between 3500 and 3000 cm$^{-1}$ correspond to stretching vibration of hydrogen bonded O-H groups. Thus, the decreased intensities of OH vibrational modes in the region of 3500-3000 cm$^{-1}$ in the spectrum of the inclusion complexes 1 and 2 are resulted from the release of included water molecules in CDs upon the inclusion of larger guest molecules. It has been well understood that the main driving force for the inclusion of a larger hydrophobic guest molecule in cyclodextrins is the release of enthalphy- and entropy-rich water molecules from the cavity.$^7,14$

In order to examine the thermal stability and stoichiometry for the inclusion complexes in solid states, we performed the thermogravimetric analysis (TGA) of CDs and the inclusion complexes 1 and 2 (Figure 2). The thermal analysis of CDs and their inclusion complexes have been well documented, and the data obtained in this work are in good agreement with those reported in the literature.$^{15}$ The TGA curves for $\beta$- and $\gamma$-CDs indicate that loss of water contents occurred up to 120 °C and $\beta$- and $\gamma$-CDs were decomposed above 290 °C (dashed lines in Figure 2a and 2b). However, the TGA curves for the inclusion complexes 1 and 2 exhibited significantly different behaviors in the course of thermal decompositions. It was found that the inclusion complexes showed at the beginning a small weight loss due to the loss of water molecules, followed by a larger weight loss corresponding to the decomposition of the pulegone and CDs.$^{16}$ From these TGA data of the inclusion complexes 1 and 2, the ratio between pulegone and CDs was estimated to be 1:1. Our TGA investigation thus supports that pulegon molecules are supramolecularly encapsulated in $\beta$- and $\gamma$-CDs with 1:1.

**Scheme 1.** Schematic illustration of the encapsulation of pulegone by cyclodextrins.

**Figure 1.** Comparison of IR spectra for inclusion complexes, CDs and pulegone. (a) 1 (top), $\beta$-CD (middle) and pulegone (bottom); (b) 2 (top), $\gamma$-CD (middle) and pulegone (bottom). Asterisk (*) represents the bands for the vibration of CO$_2$ molecules in ambient atmosphere during the measurements.
complexation.

We also studied supramolecular encapsulation of pulegone by CDs in aqueous solution state using UV-vis absorption and $^1$H NMR spectroscopy. Free $\beta$- and $\gamma$-CDs exhibited only absorption bands at ~190 nm in their aqueous solutions due to the absence of proper chromophores (dotted lines in Figure 3). However, new absorption band appeared around 260 nm in the UV-vis absorption spectra obtained from the aqueous solutions of inclusion complexes 1 and 2, as shown in Figure 3 (solid lines). This absorption is attributed by the newly incorporated chromophore (C=C=C=O) of pulegone in the inclusion complexes 1 and 2. To further substantiate the supramolecular encapsulation of pulegone by CDs in aqueous solution, the inclusion complexes 1 and 2 were also characterized by $^1$H NMR spectroscopy. In the $^1$H NMR spectra of 1 and 2 in D$_2$O, not only the resonances in the range 2.7-0.8 ppm attributed by the protons of pulegone but also the characteristic resonances of CDs were evidently observed (Figure 4). From the estimation of the area ratios, pulegone molecule is encapsulated in $\beta$- and $\gamma$-CD with 1:1 stoichiometry for 1 and 2, respectively. This stoichiometric estimation is also in good agreement with the TGA data. Therefore, the UV-vis and $^1$H NMR spectral data confidentially corroborate that water-insoluble pulegone molecules are readily dissolved in water through the supramolecular encapsulation by water-soluble host molecules, CDs.

In summary, we have investigated supramolecular encapsulation of pulegone by cyclodextrins with the aim of solubilization of water-insoluble phytochemicals for biological applications. Our investigations reveal that pulegone molecules are readily included in $\beta$- and $\gamma$-cyclodextrins with 1:1 stoichiometry, and are substantially stabilized in aqueous solution as well as solid state through the supramolecular encapsulation.

**Experimental Section**

**Measurements.** UV-vis and IR spectra were recorded on a
Hewlett-Packard 8453 diode array and a Jasco FTIR-460 Plus spectrophotometers, respectively. 1H NMR spectra were obtained on a Bruker AVANCE III 400 Micro Bay spectrometer. Matrix-assisted laser desorption ionization (MALDI-TOF) mass spectra were recorded on an Applied Biosystems Voyager DE-STR spectrometer. Elemental analyses were performed on a ThermoQuest EA 1110 analyzer. TGA data were obtained on a Perkin-Elmer Pyris 1 TGA instrument with a heating rate of 10 °C min⁻¹ under a N₂ atmosphere.

Materials. β- and γ-cyclodextrins were purchased from Aldrich. Although pulegone can be available commercially, we used our purified samples from Schizonepeta tenuifolia Briquet for the future studies on phytochemicals. A typical procedure is as follows: A mixture of phytochemicals including pulegone was extracted from grinded Schizonepeta tenuifolia Briquet (220 g) with chloroform (4.5 L) by heating at 55 °C for 12 h. The solvent of the extract was removed under reduced pressure. Pulegone was separated from the crude extract by column chromatography on silica gel using gradient eluents with hexane and chloroform (10:0 → 100:0). The purity of yielded pulegone exceeds over 99%, which was estimated by the 1H NMR spectrometry, gas chromatography (GC), and GC-Mass techniques.

Encapsulation of pulegone by cyclodextrins. To an aqueous solution of β- or γ-cyclodextrins (10⁻³ M, 10 mL), pulegone (10⁻² mmol) was directly added. The solution was stirred for 1 h at room temperature and the solvent was removed under reduced pressure. The residue was thoroughly washed with diethyl ether to remove uncomplexed pulegone, and then dried in vacuo at room temperature. For 1: Yield 95%. 1H NMR (400 MHz, D₂O): δ 5.00 (d, J = 3.6 Hz, 7H, β-CD), 3.87-3.80 (m, 21H, β-CD), 3.72-3.70 (m, 7H, β-CD), 3.60-3.50 (m, 14H, γ-CD), 3.48-3.45 (m, 1H, pulegone), 2.49-2.44 (m, 1H, pulegone), 2.25 (t, 1H, pulegone), 2.16-2.02 (m, 1H, pulegone), 1.98-1.94 (m, 1H, pulegone), 1.85 (s, 3H, pulegone), 1.76 (s, 3H, pulegone), 1.34-1.31 (m, 2H, pulegone), 0.92 (d, J = 1.6 Hz, 3H, pulegone). IR (KBr, cm⁻¹): ν 3374 (O-H), 2930, 1678 (C=O), 1645, 1456, 1395, 1355, 1329, 1270, 1230, 1164, 1087, 1036, 950, 873, 710, 590. UV-vis (H₂O, nm): λ max(log ε) 188 (2.21), 260 (1.71). MS (MALDI-TOF): m/z 1325.74 ([M+K]+) requires 1325.45. Anal. Calcd. for C₅₈H₉₆O₄₁: C, 48.83; H, 6.83. Found: C, 48.83; H, 6.73. For 2: Yield 92%. 1H NMR (400 MHz, D₂O): δ 5.04 (d, J = 3.6 Hz, 7H, β-CD), 3.88-3.76 (m, 28H, γ-CD), 3.61-3.58 (m, 7H, γ-CD), 3.55-3.50 (m, 7H, γ-CD), 2.69-2.65 (m, 1H, pulegone), 2.47-2.42 (m, 1H, pulegone), 2.16 (t, 1H, pulegone), 2.10-2.03 (m, 1H, pulegone), 1.99-1.95 (m, 1H, pulegone), 1.83 (s, 3H, pulegone), 1.75 (s, 3H, pulegone), 1.30-1.29 (m, 2H, pulegone), 0.93 (d, J = 1.6 Hz, 3H, pulegone). IR (KBr, cm⁻¹): ν 3401 (O-H), 2930, 1687 (C=O), 1645, 1456, 1402, 1365, 1329, 1164, 1079, 1036, 993, 941, 865, 770, 702, 667, 590, 530. UV-vis (H₂O, nm): λ max(log ε) 187 (2.55), 262 (1.78). Anal. Calcd. for C₅₈H₉₆O₄₁: C, 48.06; H, 6.68. Found: C, 48.12; H, 6.38.

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References and Notes

12. The results from molecular dynamics simulations of pulegone binding to permethylated β-cyclodextrins have been reported: Lipkowitz, K. B.; Coner, R.; Peterson, M. A.; Morreale, A.; Shackelford, J. J. Org. Chem. 1998, 63, 732-745.
16. In the TGA diagrams for 1 and 2, the residual amounts after thermal decomposition are attributed to the decomposed carbon-based species such as soot or tar.