Coumarin 및 Coumarin 의 Hydroxy 유도체의 초임계이산화탄소에 대한 용해도 측정에 관한 연구

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Solubility Measurements of Coumarin and Its Hydroxyl Derivatives in Supercritical Carbon Dioxide

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Introduction

Coumarin and its derivatives are widely used in pharmaceutic agents for antibiotic and antitumor activity, or vasodilatory and anticoagulant agent. Also, it is experimentally conformed that there exist more than 800 coumarin derivatives in natural plants with respect to the basic backbone 2H-1-benzopyran-2-one structure. Depending upon its backbone structure and substituted types, coumarins can be classified by hydroxycoumarin, alkoxycoumarin, alkylcoumarin, furanocoumarin, pyranocoumarin and biscoumarins. Thus, depending on a type of substituted group, coumarins in plants are extracted traditionally by various conventional organic solvents such as *n*-hexane, chloroform, ethylacetate, and methanol.

Recently Calvey et al. have reported apparent solubility threshold densities of some substituted coumarins in supercritical fluid chromatography. However, any quantitative solubility measurement studies by supercritical fluids have not reported to date in literature. Thus, we report the effect of the addition of hydroxyl group to basic coumarin structure for the solubility of substituted coumarins in supercritical carbon dioxide.

Experimental

All the agents were purchased from Sigma Chemical Co. USA(grade : above 98%). The extractor used in the present study is the flow-type(Figure 1). The cell volume was 60ml and the pressure was controlled by a gas booster and forward regulator(Tescom, 26-1700, USA). Temperature in the air-bath was controlled by PID controller. The effluent coumarin solutes dissolved in carbon dioxide is collected by a 2-step methanol trap and the extract remained in all the tubings and valves was rinsed by methanol, acetone and chloroform. The effluent flowrate was controlled at 200 ml/min at standard condition by a metering valve and every experimental runs was repeated six times. The solubility of 4-hydroxycoumarin,

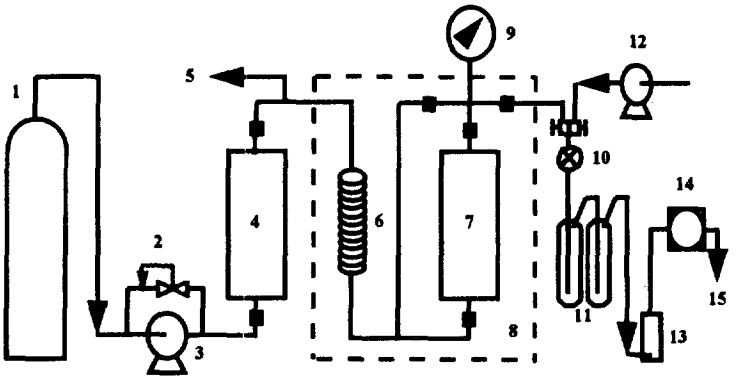

Figure 1. The schematic diagram of supercritical fluid extractor.

1 CO_2 supply	2 Back pressure regulator	3 Compressor	4 Reserver
5 Vent	6 Preheater	7 Extractor	8 Air bath
9 Heise gauge	10 Metering valve	11 Cold trap	12 Washing pump
13 Flow meter	14 Total mass meter	15 Vent	

7-hydroxycoumarin and 6,7-dihydroxycoumarin were measured at 308.15, 313,15 and 323.15 K, respectively and at each isotherm for 8.5, 10, 15, 20 and 25MPa. However, for coumarin the solubility is measured limitedly at 308.15 and 313.15K to prevent partial melting during experiment since coumarin has very low melting point compared to the other derivatives. Since the coumarin showed extremely higher solubility than the other derivatives, the extract of coumarin was collected until the consumed carbon dioxide become 5 *l* and each of the extracts of 4-, 7-, 6,7-dihydroxycoumarin, 40 *l*.

Each collected extract was dried in a vacuum evaporator to be securely free from solvents in the cold trap. All the samples were diluted by methanol and injected to YMC-Pack, ODS-A column(250 x 4.6 mm, s-5 μm, YMC Inc., Japan). HPLC is equipped constametric 3000 pump, spectromonitor 3100 variable wavelength detector fixed at 280nm(MILTON ROY, USA) and chromatocorder 12. Solvent used with HPLC was a mixture of 40 by 60 ratio of 5% formic acid and methanol.

Results and Discussion

Measured solubilities of coumarin, 4-hydroxycoumarin, 7-hydroxycoumarin and 6,7-dihydroxycoumarin are in Figure 2. Within experimental ranges of supercritical pressure and temperature, coumarin shows much higher solubility in carbon dioxide than both the case of 4- and 7-hydroxycoumarin. However, we could not detect the solubilized 6,7-dihydroxycoumarin in carbon dioxide. At 313.15K, when we compare the solubility of coumarin from 8.5MPa to 25MPa, there was approximately five times enhancement of

solubility with increasing pressure. However there was about thirty times increase of solubility with increasing pressure for the cases of 4- and 7-hydroxycoumarin. In contrast to the case of hydroxylated coumarin derivatives which have significant hydrogen bonding effect, coumarin has low polarity and show higher solubility even at low density of carbon dioxide.

For other solutes such as hydrocarbons or naphthol, derivatives made by the addition of hydroxyl group affect significantly the solubility behaviors in supercritical carbon dioxide. In the case of coumarin, monohydroxycoumarins such as 4- or 7-hydroxycoumarin showed about 10^3 times lower solubilities than coumarin and dihydroxicoumarin(6,7-dihydroxycoumarin) was almost insoluble in carbon dioxide. Calvey et al. and Miachi et al. report that the solubility of dihycroxycoumarin stay beyond the HPLC-UV detectable range(10^{-12}). Since carbon dioxide is nonpolar, 6,7-dihydroxycoumarin which has relatively strong hydrogen bonding force showed almost no solubility.

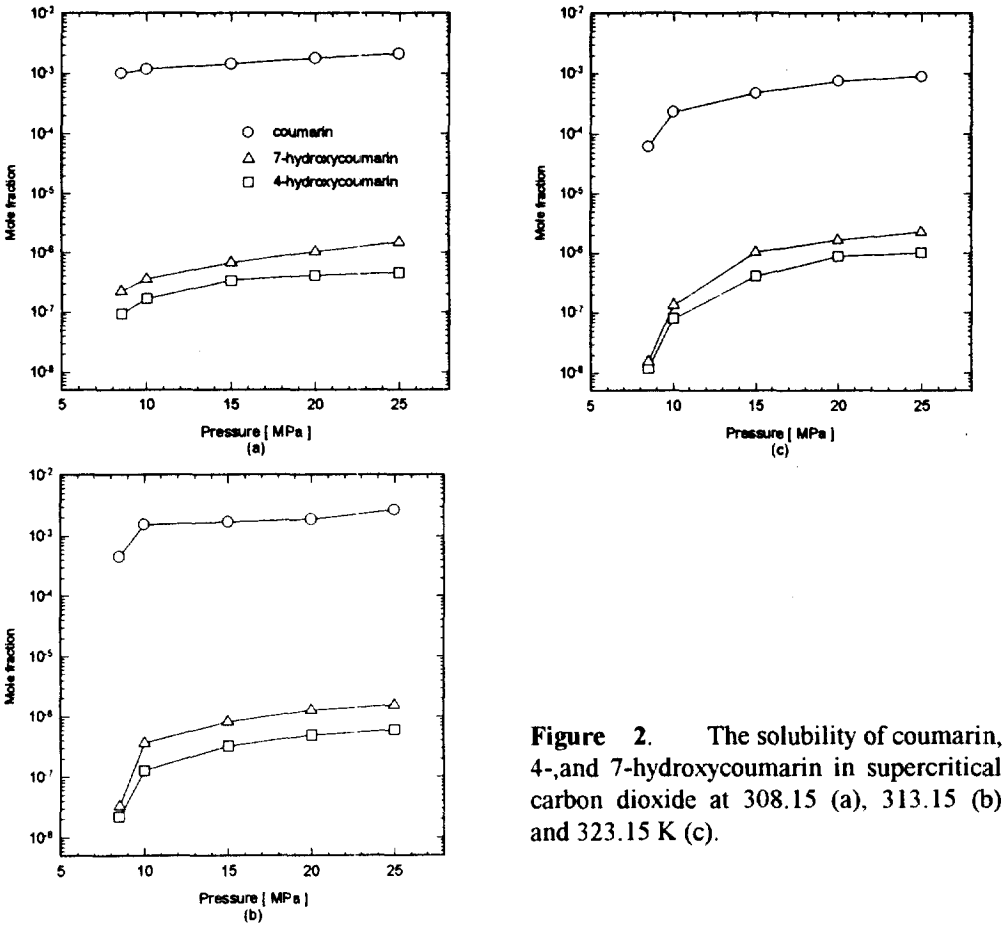

Figure 2. The solubility of coumarin, 4-,and 7-hydroxycoumarin in supercritical carbon dioxide at 308.15 (a), 313.15 (b) and 323.15 K (c).

We also found that 7-hydroxycoumarin which is one of the isomer of 4-hydroxycoumarin showed twice times higher solubility than 4-hydroxycoumarin over the entire experimental range. As the others' works determined the solubility variations of isomers in carbon dioxide for other compounds, solubilities of isomers in carbon dioxide were frequently independent on physical properties of isomers such as melting point, boiling point and the position of functional group such as o-, m- and p-substitution.

The solubilities of monohydroxycoumarins in the pressure range of 10-15 MPa are increasing with increasing temperature. Based on this result, the solubility of monohydroxycoumarin is initially proportional to pressure and the density of carbon dioxide at mild supercritical conditions, however, beyond 10MPa, the solubilities tend to increase with increasing temperature regardless of the density of carbon dioxide. Compared to coumarin, the solubilities of 4- and 7-hydroxycoumarin tend to increase with increasing the density of carbon dioxide.

Conclusion

In summary, we found that coumarin has higher solubility in supercritical carbon dioxide than any other hydroxy-substituted coumarins. The single addition of hydroxyl group tend to decrease the solubility about 10^3 times. Depending upon the position of hydroxyl group to the basic coumarin structure, monohydroxycoumarin isomers show different polarity increase and thus different solubilities in supercritical carbon dioxide. The substituted coumarins by the addition of two hydroxyl groups such as 6,7-dihydroxycoumarin showed insolubility in carbon dioxide.

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