Solubility of Podophyllotoxin in Six Organic Solvents from (283.2 to 323.2) K

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The solubility of podophyllotoxin (furo[3′,4′:6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one, 5,8,8a,9-tetrahydro-9-hydroxy-5-(3,4,5-trimethoxyphenyl), (5*R*,5a*R*,8a*R*,9*R*)) in ethanol, methanol, acetone, ethyl acetate, propan-2-ol, and butan-1-ol from (283.2 to 323.2) K was measured under atmospheric pressure. The solubility of podophyllotoxin in these solvents increases with increasing temperature. The solubility values were correlated with the Apelblat equation.

Introduction

Podophyllotoxin (furo[3′,4′:6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one, 5,8,8a,9-tetrahydro-9-hydroxy-5-(3,4,5-trimethoxyphenyl), (5*R*,5a*R*,8a*R*,9*R*); $C_{22}H_{22}O_8$; molecular weight 414.41; CAS Registry Number 518-28-5; Figure 1) is a naturally occurring aryltetralin lignan that exists widely in plant species of the subfamily Podophylloideae (Berberidaceae).[1] Since its first isolation from podophyllin by Podwyssotzki in 1880, podophyllotoxin has been found to have potential antitumor effects.[2,3] Modern pharmacological studies have demonstrated that the cytotoxicity of podophyllotoxin is associated with inhibiting DNA topoisomerase II by stabilizing the covalent topo II DNA cleavable complex.[4] Although it can not be applied directly in clinical use as an antitumor agent for its unacceptable gastrointestinal toxicity, podophyllotoxin now plays a role as an important starting material for the development of new therapeutic agents based on structural modifications. Two semisynthetic glucoconjugate analogues, etoposide and teniposide,[5] have already been developed as important anticancer agents for clinical usage and trigged the synthesis of podophyllotoxin derivatives with various modifications, which has caused a large demand for its production. Nowadays, compared to other methods, such as plant cell culture and endogenesis epiphyte zymolysis, extraction and crystallization from plants is still the most efficient and most economical way to obtain pure podophyllotoxin, [6] where organic solvents such as ethanol, methanol, acetone, etc. are frequently used. Therefore, the solubility data of podophyllotoxin in different organic solvents will become an important reference in the extraction process studies.

In the current project, the solubility of podophyllotoxin in six organic solvents, ethanol, methanol, acetone, ethyl acetate, propan-2-ol, and butan-1-ol. over the temperature range of (283.2 to 323.2) K was measured by HPLC, and the results were fitted with the modified Apelbat equation.

Experimental Section

Reagents and Apparatus. Podophyllotoxin was supplied by Guanyu Biology Technology Co. (Xi'an, China) with minimum purity of 99.0 %. All organic solvents used were analytical purity grade and obtained from Shuanglin Chemical Reagent Factory (Hangzhou, China). Redistilled deionized water was used throughout. The solvent of the mobile phase was Burdick and Jackson ACS/HPLC Certified. A THZ-C shaker was supplied by Taicang Laboratorial Equipment Factory (Hangzhou, China), and an Elite HPLC instrument P230II coupled with a UV230II detector was used for analysis of samples.

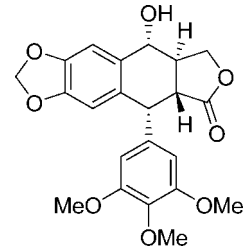

Figure 1. Chemical structure of podophyllotoxin.

Sample Preparation. Excess amounts of podophyllotoxin were added to 5 mL of six organic solvents (ethanol, methanol, acetone, ethyl acetate, propan-2-ol, butan-1-ol) with their temperatures ranging from (283.2 to 323.2) K. The temperature was controlled by a thermostat (uncertainty of ± 0.1 K) in the shaker. The suspended solution was kept shaken for 24 h in the shaker. After attaining equilibrium, the supernatant liquid was withdrawn and filtered through a 0.45 μm membrane. The filtered samples were poured into a 50 mL volumetric flask and diluted to a fixed volume for HPLC analysis. The sampling process before dilution was operated at the same temperature in the shaker. Each measurement was repeated three times.

Sample Analysis. To determine the composition of podophyllotoxin, an HPLC system mentioned above was used with its wavelength of detection set at 290 nm. All chromatographic separations were performed at 30 °C (uncertainty of ± 0.1 °C) using a Phenomenex chromatography column (Gemini 5 μ, C18, 110A, 250 × 4.60 mm, 5 μm). The mobile phase was methanol (1) + water (2) with a volume fraction of $\phi_1 = 0.65$ and its flow rate at 1.0 mL·min^{-1} and injection volume being 20 μL. The calibration curve for estimation of podophyllotoxin was established by using the standard solutions in the appropriate concentration range. The measuring relative expanded uncertainty (U_{rel}) is 2 % with a 95 % confidence level (coverage factor, $k = 2$).

Result and Discussion

The solubility values of podophyllotoxin in ethanol, methanol, acetone, ethyl acetate, propan-2-ol, and butan-1-ol were measured with their data summarized in Table 1. The solubility of podophyllotoxin was a function of temperature, and solubility increased with temperature in these six pure organic solvents. The solubility

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Table 1. Solubility c (mol·L^{-1}) of Podophyllotoxin in Ethanol (1), Methanol (2), Acetone (3), Ethyl Acetate (4), Propan-2-ol (5), and Butan-1-ol (6)

T/K	c_1	$c_1-c_1^c$	c_2	$c_2-c_2^c$	c_3	$c_3-c_3^c$	c_4	$c_4-c_4^c$	c_5	$c_5-c_5^c$	c_6	$c_6-c_6^c$
283.2	0.0583 ± 0.0006	−0.0029	0.0393 ± 0.0006	−0.0024	0.0971 ± 0.0006	0.0041	0.0904 ± 0.0004	−0.0019	0.0509 ± 0.0006	−0.0019	0.0401 ± 0.0004	−0.0006
293.2	0.0814 ± 0.0002	0.0040	0.0518 ± 0.0005	−0.0005	0.1039 ± 0.0004	−0.0068	0.1046 ± 0.0003	0.0080	0.0582 ± 0.0004	0.0003	0.0538 ± 0.0003	0.0015
303.2	0.0960 ± 0.0007	0.0016	0.0577 ± 0.0005	−0.0054	0.1536 ± 0.0003	0.0063	0.1068 ± 0.0004	−0.0010	0.0735 ± 0.0006	0.0052	0.0641 ± 0.0009	−0.0008
313.2	0.1060 ± 0.0007	−0.0053	0.0718 ± 0.0004	−0.0017	0.2180 ± 0.0005	0.0014	0.1258 ± 0.0008	−0.0017	0.0787 ± 0.0009	−0.0073	0.0781 ± 0.0003	0.0000
323.2	0.1295 ± 0.0004	0.0020	0.0787 ± 0.0007	−0.0042	0.3498 ± 0.0005	0.0026	0.1615 ± 0.0008	0.0032	0.1158 ± 0.0002	0.0013	0.0915 ± 0.0006	0.0002

of podophyllotoxin in these solvents decreased in the order: acetone > ethyl acetate > ethanol > propan-2-ol > butan-1-ol > methanol. These experimental data could be regressed by eq 1 for each solvent. Among these solvents, the solubility of podophyllotoxin in acetone increased most significantly with the increasing temperature. Podophyllotoxin dissolved more easily in acetone and ethyl acetate than lower alcohols, which can be explained by the structure similarity between the solvent and solute due to the carbonyl group, corresponding to the empirical rule "like dissolves like" to some extent.

The experimental solubility of podophyllotoxin increases with an increase in temperature (Figure 2). Thus the solubility of podophyllotoxin as a function of temperature in pure solvents is correlated by the modified Apelbat equation.[7−11]

$$\ln(c/\mathrm{mol\cdot L^{-1}}) = A + \frac{B}{T/\mathrm{K}} + C\ln(T/\mathrm{K}) \qquad (1)$$

where A, B, and C are parameters; T is the absolute temperature; and c is the mole fraction solubility of podophyllotoxin. The experimental solubility values have been correlated with eq 2 by the least-squares method (see below). The regressed values of the parameters A, B, and C for the modified Apelbat equation are listed in Table 2 together with the root-mean-square deviation (rmsd), namely, standard deviation, which is defined as the following

$$\mathrm{rmsd} = \sqrt{\frac{\sum_{i=1}^{N}(c_i^c - c_i)^2}{N}} \qquad (2)$$

where N is the number of the experiment points; c_i and c_i^c denote the experimental and calculated values of the solubility, respectively. The calculated solubility of podophyllotoxin at different temperatures in six solvents, methanol, ethanol, ethyl acetate, acetone, propan-2-ol, and butan-1-ol, accords with the experimental data, which was also shown in Figure 2.

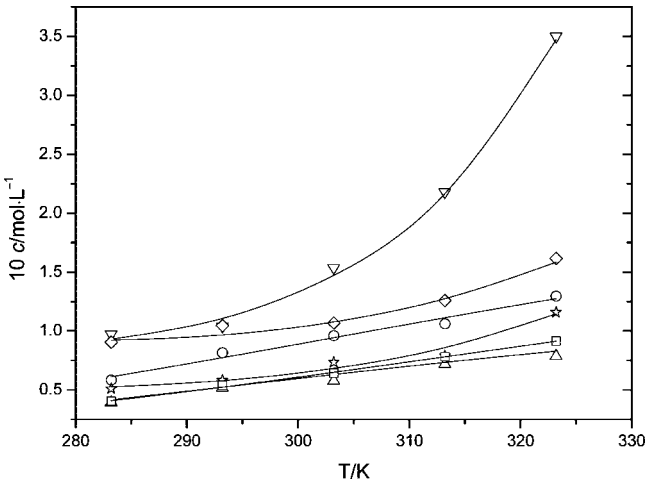

Figure 2. Experimental solubility of podophyllotoxin at different temperatures in six solvents: ○, ethanol; △, methanol; ▽, acetone; ◇, ethyl acetate; ☆, propan-2-ol; □, butan-1-ol. The corresponding lines are from the calculated values by eq 1.

Table 2. Parameters of Equation 1 for Podophyllotoxin in Solvents

solvent	A	B	C	10^3 rmsd (mol·L^{-1})
ethanol	130.336	−7406	−18.947	3.44
methanol	152.775	−8347	−22.401	3.33
acetone	−739.523	30660	111.383	4.69
ethyl acetate	−404.726	17080	60.578	4.05
propan-2-ol	−472.63	19660	70.892	4.13
butan-1-ol	114.431	−6850	−16.550	0.81

From Tables 1 and 2, it could be seen that the calculated solubilities showed good agreement with experimental values, indicating the modified Apelblat equation could be applied to correlate the solubility data of podophyllotoxin in the six organic solvents. The results show the solubility of podophyllotoxin in these solvents increases with an increase of temperature, which was relevant with the movement among the molecules. The experimental solubility and the modified Apelblat equation with the parameters might be used as essential data in purification and crystallization of podophyllotoxin.

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