1366 *J. Chem. Eng. Data* **2007,** *52,* 1366−1368

# Solubilities of Betulin in Fourteen Organic Solvents at Different Temperatures

**Dan Cao, Guoling Zhao, and Weidong Yan***

Department of Chemistry, Zhejiang University, Hangzhou 310027, PRC

The solubilities of betulin in methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, methyl formate, ethyl formate, methyl acetate, ethyl acetate, chloroform, dichloromethane, cyclohexane, and acetone were measured at $T$ = (278.2, 288.2, 298.2, and 308.2) K. The solubilities of betulin in selected organic solvents increase with temperature. A three-parameter equation was used to correlate the experimental data. These data of solubility can be used to guide the processes of crystallization in industry.

## Introduction

Betulin (Figure 1, CASRN 473-98-3, FW 442.7, white in color) and betulinic acid are natural products. Betulin exists in large amounts in the outer bark of the white birch.[1] Betulinic acid and many of its derivatives exhibit great promise as bioactive agents for the treatment of HIV infection and human melanoma. Betulinic acid can be easily prepared from the more plentiful precursor betulin in a simple two-step process. So the abundant availability of betulin from natural resources gives rise to research interest in its applications.[2,3]

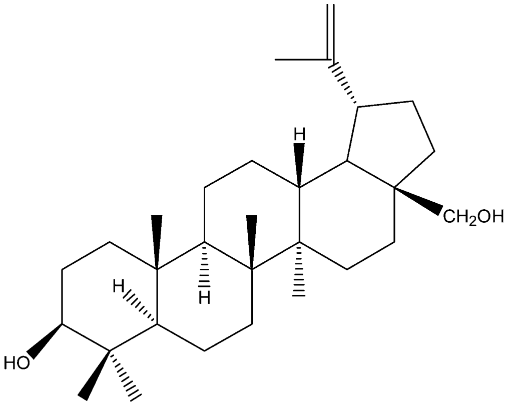
Betulin was extracted from the outer bark of the white birch by an ethanol + water mixture. The crude betulin (0.5 to 0.6 mass fraction) was purified by recrystallizing in a mixed solvent of chloroform + methanol. To reduce the loss of betulin in the mother liquor and increase the productivity of purification, the studies of phase equilibrium, especially of solubility data, are important. They are used to guide the processes of extraction and crystallization in industry. The solubility data of betulin in pure organic solvents have not been found in the literature.

Normally there are three methods to measure the concentration of a saturated solution of solute dissolved in solvent: laser technique,[4] gravimetrical method,[5] and analytical method.[6] The laser technique is used to obtain the data quickly, but it is not easy to check whether the solid−liquid equilibrium is reached, especially for systems that can emulsify. The gravimetrical method is simple and reliable, but it is usually used to measure systems where the solubility is high. The analytical method can be used to determine the concentration of a saturated solution by chemical methods of analysis, e.g., the HPLC method and the UV method. It is useful to measure systems where the solubility is very low. The solubilities of betulin are too low to be measured by the gravimetrical method. As a result, the analytical method was employed in this work.

The solubilities of betulin in methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, methyl formate, ethyl formate, methyl acetate, ethyl acetate, chloroform, dichloromethane, cyclohexane, and acetone were measured at $T$ = (278.2, 288.2, 298.2, and 308.2) K by the analytical method. A three-parameter equation was used to correlate the experimental data.

## Experimental Section

***Materials.*** The crude powder of betulin (0.92 mass fraction) was supplied by Skyherb Natural Product Co., Ltd. (China). The crude betulin was dissolved in a mixture of methanol and chloroform in a volume ratio of 1:1, refluxed about 3 h, and recrystallized at room temperature. The betulin was dried in a vacuum oven at $T$ = 348.2 K for 24 h and stored in a desiccator to avoid absorbing the water. The purity was higher than 0.995 mass fraction, determined by HPLC (Shimadzu LC-10AD).[7] The reference standard of Betulin, with a purity of > 0.98 mass fraction, was purchased from Sigma-Aldrich Chemical Corporation (USA).



Betulin ($C_{30}H_{50}O_2$)

**Figure 1.** Molecular structure of betulin.

All of the organic solvents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (China). The solvents were dehydrated with molecular sieves (3 to 4) Å before use. The purities of the organic solvents, determined by gas chromatography, were > 0.998 mass fraction.

***Sample Preparation.*** Glass-stoppered test tubes (10 mL) were used to prepare saturated solutions (about 8.0 mL) of betulin with excess solid solute in organic solvents. The test tubes were stoppered and sealed with Parafilm and Teflon tape to prevent evaporation of solvents. Then the test tubes were placed in a constant temperature thermostatic bath (PolyScience chiller circulator 9501, USA) with a temperature stability of ± 0.01 K and a temperature uncertainty of ± 0.25 K. The test tubes were allowed to settle about 36 to 48 h to ensure equilibrium. For each test tube, three samples of approximately (0.2 to 0.6) mL were withdrawn from the clear saturated solution using preheated glass syringes. The glass syringe with a saturated solution was weighted using a Sartorius Type 1712 analytical balance with an uncertainty of ± 0.01 mg. The needle was closed with

---

* To whom correspondence should be addressed. Tel: 0086 571 87951430. Fax: 0086 571 87951895. E-mail: yanweidong@zju.edu.cn.

10.1021/je700069g CCC: $37.00

Table 1. Experimental Data of Solubilities for Betulin in Different Solvents at $T$ = (278.2, 288.2, 298.2, and 308.2) K[a]

| $T$/K | $10^3 m_i$/mol·kg$^{-1}$ | $10^4 x_i$ | $T$/K | $10^3 m_i$/mol·kg$^{-1}$ | $10^4 x_i$ |
|---|---|---|---|---|---|
| | Methanol | | | Ethanol | |
| 278.2 | 5.60 ± 0.07 | 1.79 ± 0.02 | 278.2 | 12.60 ± 0.2 | 5.79 ± 0.08 |
| 288.2 | 7.74 ± 0.15 | 2.48 ± 0.05 | 288.2 | 14.31 ± 0.2 | 6.58 ± 0.10 |
| 298.2 | 10.24 ± 0.07 | 3.28 ± 0.02 | 298.2 | 19.50 ± 0.4 | 8.98 ± 0.18 |
| 308.2 | 12.41 ± 0.57 | 3.97 ± 0.18 | 308.2 | 24.67 ± 0.2 | 11.33 ± 0.09 |
| | 1-Propanol | | | 1-Butanol | |
| 278.2 | 18.90 ± 0.24 | 11.33 ± 0.14 | 278.2 | 24.47 ± 0.77 | 18.07 ± 0.83 |
| 288.2 | 21.26 ± 0.51 | 12.74 ± 0.31 | 288.2 | 28.81 ± 0.34 | 21.30 ± 0.25 |
| 298.2 | 24.42 ± 0.36 | 14.63 ± 0.21 | 298.2 | 32.31 ± 0.01 | 23.85 ± 0.01 |
| 308.2 | 26.67 ± 0.44 | 16.00 ± 0.19 | 308.2 | 36.76 ± 0.56 | 27.13 ± 0.42 |
| | 1-Pentanol | | | 1-Hexanol | |
| 278.2 | 10.80 ± 0.59 | 9.50 ± 0.52 | 278.2 | 7.25 ± 0.06 | 7.44 ± 0.06 |
| 288.2 | 13.69 ± 0.37 | 12.04 ± 0.33 | 288.2 | 8.27 ± 0.03 | 8.46 ± 0.03 |
| 298.2 | 16.29 ± 0.51 | 14.32 ± 0.45 | 298.2 | 10.00 ± 0.11 | 10.20 ± 0.11 |
| 308.2 | 19.45 ± 0.78 | 17.13 ± 0.69 | 308.2 | 12.46 ± 0.36 | 12.73 ± 0.37 |
| | Methyl Formate[b] | | | Ethyl Formate | |
| 278.2 | 3.12 ± 0.10 | 1.87 ± 0.06 | 278.2 | 4.07 ± 0.01 | 3.01 ± 0.01 |
| 283.2 | 3.53 ± 0.14 | 2.12 ± 0.08 | 288.2 | 4.51 ± 0.03 | 3.33 ± 0.03 |
| 288.2 | 4.29 ± 0.06 | 2.57 ± 0.04 | 298.2 | 4.89 ± 0.04 | 3.16 ± 0.19 |
| 298.2 | 5.27 ± 0.25 | 3.16 ± 0.2 | 308.2 | 5.34 ± 0.02 | 3.95 ± 0.01 |
| | Methyl Acetate | | | Ethyl Acetate | |
| 278.2 | 8.09 ± 0.02 | 5.98 ± 0.01 | 278.2 | 10.94 ± 0.95 | 9.63 ± 0.84 |
| 288.2 | 9.53 ± 0.02 | 7.05 ± 0.01 | 288.2 | 17.65 ± 0.07 | 15.50 ± 0.06 |
| 298.2 | 10.78 ± 0.05 | 7.97 ± 0.04 | 298.2 | 23.52 ± 0.57 | 20.66 ± 0.51 |
| 308.2 | 12.40 ± 0.19 | 9.17 ± 0.14 | 308.2 | 34.72 ± 0.50 | 30.47 ± 0.44 |
| | Chloroform | | | Dichloromethane[b] | |
| 278.2 | 21.85 ± 0.57 | 26.02 ± 0.68 | 278.2 | 9.250 ± 0.003 | 7.90 ± 0.03 |
| 288.2 | 28.39 ± 0.55 | 33.77 ± 0.66 | 283.2 | 9.85 ± 0.06 | 8.37 ± 0.05 |
| 298.2 | 36.77 ± 0.06 | 43.70 ± 0.08 | 288.2 | 10.57 ± 0.07 | 8.97 ± 0.06 |
| 308.2 | 43.03 ± 0.30 | 51.11 ± 0.43 | 298.2 | 11.67 ± 0.14 | 9.91 ± 0.12 |
| | Cyclohexane[c] | | | Acetone | |
| 283.2 | 0.200 ± 0.001 | 0.168 ± 0.008 | 278.2 | 9.59 ± 0.07 | 5.56 ± 0.03 |
| 288.2 | 0.322 ± 0.006 | 0.270 ± 0.010 | 288.2 | 14.95 ± 0.41 | 8.66 ± 0.20 |
| 298.2 | 0.755 ± 0.006 | 0.634 ± 0.021 | 298.2 | 27.26 ± 0.66 | 15.78 ± 0.38 |
| 308.2 | 1.944 ± 0.004 | 1.667 ± 0.027 | 308.2 | 39.27 ± 0.33 | 22.73 ± 0.19 |

[a] Expanded uncertainties (±) were calculated using standard deviation, SD, × coverage factor $k$; $k = 2$. [b] $T$ = (278.2, 283.2, 288.2, and 298.2) K. [c] $T$ = (283.2, 288.2, 298.2, and 308.2) K.

silicon rubber to prevent evaporation of solvents during the weighing procedure. To prevent precipitation, the saturated solution was injected into the 10 mL volumetric flask immediately. Subsequently, the mass of the glass syringe with the remaining solution was weighed. Then the mass of saturated solutions which were put into volumetric flasks was calculated. The solutions of samples used for analysis were diluted to the mark with methanol.

An accurately weighed quantity of betulin RS was dissolved in methanol to obtain a reference standard solution having a known concentration of about 0.25 mg·mL$^{-1}$.

***Chromatographic Conditions.*** The solubility was determined using HPLC (Shimadzu Corporation, Kyoto, Japan) consisting of a degasser (DGU-4A), a solvent delivery module (LC-10AT), and a UV detector (SPD-10A). Data were acquired using the N2000 Chromatographic Data System (Zheda Information and Technologies Ltd., Hangzhou, China).

The analysis was performed on a Diamonsil $C_{18}$ column (250 mm × 4.6 mm, 5 $\mu$m). The optimum separation of HPLC was carried out with a mobile phase composed of acetonitrile and water in a volume ratio of 92:8 at a flow rate of 1.0 mL·min$^{-1}$. The injected volumes of sample and reference standard solutions were 20 $\mu$L. The detective wavelength was set at 210 nm. All chromatograph procedures were performed at room temperature.[7]

## Results and Discussion

The solubilities of betulin in selected solvent are very low. High-performance liquid chromatography was chosen to determine the concentration of a saturated solution of betulin in the organic solvent. To check the reliability of the experimental method, known masses of betulin were completely dissolved in methanol and the concentrations of solution were measured by HPLC. The average relative uncertainty was 2.6 % ($n = 6$).

The solubilities of betulin in methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, methyl formate, ethyl formate, methyl acetate, ethyl acetate, chloroform, dichloromethane, cyclohexane, and acetone were determined at different temperatures and are listed in Table 1. Molalities, $m_i$ (mol·kg$^{-1}$), and mole fraction, $x_i$, values in Table 1 are the average values taken from two test tubes and three samples for each tube with the same solvent. The expanding uncertainty (±) for each data point is given in Table 1. The experimental value of betulin in cyclohexane at $T$ = 278.2 K is absent because the melting point of cyclohexane is 279.65 K. Furthermore, the data of betulin in dichloromethane and methyl formate at $T$ = 308.2 K are absent because the boiling point of methyl formate is 305.2 K and with

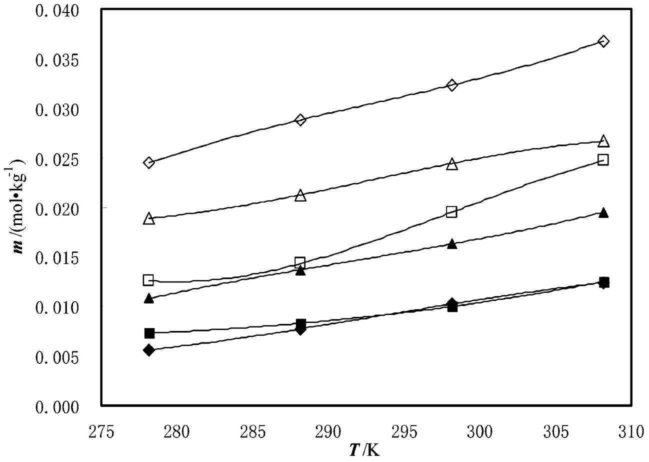


**Figure 2.** Solubility of betulin in different alcohol solvents: ◆, methanol; □, ethanol; △, 1-pentanol; ◇, 1-butanol; ▲, 1-propanol; ■, 1-hexanol; line, calculated by eq 4 (parameters taken from Table 2).

**Table 2. Parameters of Equation 4 for Betulin in Different Solvents**

| solvent | $a$ | $b$/K | $c$ | $10^4$rmsd |
|---|---|---|---|---|
| methanol | 269.49 | −14110.13 | −40.403 | 0.02 |
| ethanol | −408.16 | 15877.85 | 61.053 | 0.23 |
| 1-propanol | 33.58 | −2613.37 | −5.503 | 0.11 |
| 1-butanol | −11.37 | −783.64 | 1.400 | 0.35 |
| 1-pentanol | 93.33 | −5797.89 | −14.115 | 0.10 |
| 1-hexanol | −343.57 | 13438.14 | 51.181 | 0.02 |
| methyl formate | 24.38 | −3405.06 | −3.684 | 0.03 |
| ethyl formate | 7.91 | −1343.82 | −1.987 | 0.01 |
| methyl acetate | 24.38 | −1857.44 | −2.235 | 0.05 |
| ethyl acetate | −92.06 | 950.24 | 14.516 | 0.29 |
| chloroform | 206.01 | −10935.87 | −30.676 | 0.46 |
| dichloromethane | 103.22 | −5591.27 | −16.039 | 0.03 |
| cyclohexane | −511.51 | 15457.58 | 78.983 | 0.01 |
| acetone | 30.65 | −5157.51 | −3.486 | 0.59 |

dichloromethane, which has a boiling point of 313.2 K, evaporation occurred at $T$ = 308.2 K. So the solubilities of betulin in cyclohexane, dichloromethane, and ethyl formate were determined at $T$ = 288.2 K.

According to the solid−liquid phase equilibrium theory, the relationship between solubility and temperature is described as[8]

$$\ln\left(\frac{1}{\gamma_x x}\right) = \frac{\Delta_{\text{fus}}H}{RT_{\text{t}}}\left(\frac{T_t}{T} - 1\right) - \frac{\Delta C_p}{R}\left(\frac{T_{\text{t}}}{T} - 1\right) + \frac{\Delta C_p}{R}\ln\frac{T_{\text{t}}}{T} \quad (1)$$

where $\gamma_x$ is the activity coefficient of betulin on a mole fraction basis; $x$ is the mole fraction solubility of betulin; $\Delta_{\text{fus}}H$ is the enthalpy of fusion of betulin; $\Delta C_p$ is the change of the heat capacity; $T$ is the absolute temperature; $T_{\text{t}}$ is the triple-point temperature of betulin; and $R$ is the gas constant. For regular solutions, the activity coefficient is given by[9]

$$\ln \gamma_x = A + \frac{B}{T/\text{K}} \quad (2)$$

where $A$ and $B$ are constants. Introducing $\gamma_x$ from eq 2 into eq 3 and subsequent rearrangement results in

$$\ln x = \left[\frac{\Delta_{\text{fus}}H}{RT_{\text{t}}} + \frac{\Delta C_p}{R}(1 + \ln T_{\text{t}}) - A\right] - \left[B + \left(\frac{\Delta_{\text{fus}}H}{RT_{\text{t}}} + \frac{\Delta C_p}{R}\right)T_{\text{t}}\right]\frac{1}{T} - \frac{\Delta C_p}{R}\ln T \quad (3)$$

Equation 3 can be written as

$$\ln x = a + \frac{b}{T/\text{K}} + c\ln T/\text{K} \quad (4)$$

where $a$, $b$, and $c$ are empirical parameters. The experimental data of mole fraction solubility in Table 1 were correlated with eq 4. The values of the three parameters $a$, $b$, and $c$ together with the root mean square deviations (rmsd's) are also listed in Table 2. The rmsd's are defined as

$$\text{rmsd} = \left[\frac{1}{n}\sum_{i=1}^{n}(x_{\text{i,calcd}} - x_{\text{i,exptl}})^2\right]^{1/2} \quad (5)$$

where $x_{\text{calcd}}$ is the solubility calculated by eq 4 using the parameters in Table 2; $x_{\text{exptl}}$ is the experimental value of mole fraction solubility of betulin; and $n$ is the number of experimental points.

The data for solubility of betulin in different alcohol solvents were plotted as shown in Figure 2. From Table 1 and Figure 2, some conclusions could be obtained: (1) The solubility of betulin in pure organic solvents increases with increasing temperature. (2) The solubility of betulin in alcohol solvents decreases in the order 1-butanol > 1-propanol > ethanol > 1-pentanol > 1-hexanol > methanol. (3) The solubility of betulin in ester solvents decreases in the order ethyl acetate > methyl acetate > ethyl formate ≈ methyl formate.

## Literature Cited

(1) O'Connell, M. M.; Bentley, M. D.; Campell, C. S. Betulin and lupeol in bark from four white-barked birches. *Phytochemistry* **1988**, *27*, 2175−2176.
(2) Soler, F.; Poujade, C.; Evers, M. Betulinic Acid Derivatives: A New Class of Specific Inhibitors of Human immunodeficiency Virus Type 1 Entry. *J. Med. Chem.* **1996**, *39*, 1069−1083.
(3) Kim, D. S. H. L.; Pezzuto, J. M.; Pisha, E. Synthesis of betulin acid derivatives with activity against human melanoma. *Bioorg. Med. Lett.* **1998**, *8*, 1707−1712.
(4) Li, Q. S.; Li, Z.; Wang, S. Solubility of 4-(3,4-Dichlorophenyl)-1-tetralone in Some Organic Solvents. *J. Chem. Eng. Data* **2007**, *52*, 151−153.
(5) Fu, R. R.; Yan, W. D.; Zhu, M. Solubility and Density of the Disodium Salt hemiheptahydrate of Ceftriaxone in Water + Methanol Mixtures. *J. Chem. Eng. Data* **2004**, *49*, 262−263.
(6) Hefter, G. T.; Tomkins, R. P. T. *The Experimental Determination of Solubilities*; John Wiley: Chichester, 2003; p 260.
(7) Zhao, G. L.; Yan, W. D.; Cao, D. Simultaneous determination of betulin and betulinic acid in white birch bark using RP-HPLC. *J. Pharm. Biomed. Anal.* **2007**, *43*, 959−926.
(8) Prausnitz, J. M.; Lichtenthaler, R. N.; Gomes de Azevedo, E. *Molecular Thermodynamics of Fluid-Phase Equilibria*, 3rd ed.; Prentice Hall: New York, 1999; p 641.
(9) Kondepudi, D. K.; Prigogine, I. *Modern Thermodynamics*; John Wiley: Chichester, England, 2002.

Received for review February 3, 2007. Accepted April 6, 2007.

JE700069G