Solubilities of 2,4-Dinitro-L-phenylalanine in Monosolvents at (273.15 to 368.15) K

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The solubilities of amino acid, 2,4-dinitro-L-phenylalanine in monosolvents water, methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, acetone, ethyl acetate, and 1,2-dichloroethane were measured from (273.15 to 368.15) K at atmospheric pressure using the synthetic method. The solubility was determined by a laser monitoring observation technique. The experimental solubilities were regressed by the modified Apelblat equation with the relative deviation less than 2.5 %.

Introduction

The solubility of an uncombined substance is an important property, characterizing and quantitatively defining certain inherent properties. It depends, however, not only upon properties of the liquid phase but also upon those of the substances in the solid state.[1] Recent advances in the biochemical industry draw much attention to the development of more sophisticated and efficient processes for the separation, concentration, and purification of biomolecules. Amino acids are the simplest biomolecules, and the solubility of unnatural amino acids that can be utilized as food additives and constituents of pharmaceutical products is of great importance.

2,4-Dinitro-L-phenylalanine is a special one because its derivatives of three stereoisomers of diaminopimelic acid (DAP), which was thought to be mainly synthesized by bacteria incorporated in the peptidoglycan of bacterial cell walls,[2] were successfully separated with HPLC,[3] and the phenylalanine residue in protein could lead to a dramatic increase in both solubility and stability for its hydrophobicity.[4] However, pure 2,4-dinitro-L-phenylalanine has not been obtained yet in the literature. It is an important intermediate in our synthesis of indoline-2-carboxylic acid. It was obtained from the successive nitration of L-phenylalanine, and to find a suitable solvent for its recrystillization, its solubility was measured in different solvents including water, methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, acetone, 1,2-dichloroethane, and ethyl acetate. They may be useful for the separation and scale-up.

There are two methods to measure solubility: the analytic method and the synthetic method.[5] In the analytic method, all the materials are stirred together at a given temperature for a certain time, and then the upper solution is analyzed after the stirring stops for some time and the concentration of solute considered as the solubility at the temperature. In the synthetic method, the amount of the materials is known, and the solubility can be determined by observing the disappearance of the solid phase. Recently, the laser technique has been widely used to monitor the solution of the solid and get the solubility data.[6]

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Experimental Methods

Chemicals. 2,4-Dinitro-L-phenylalanine, not commercially available and synthesized from the nitrition of L-phenylalanine following a procedure similar to that of Almog[7] employing urea nitrate as the nitrating agent, was twice recrystillized from 1-propanol with a melting point of (191.5 to 192.4) °C (dec.). The 2,4-dinitro-L-phenylalanine thus obtained had a mass fraction purity greater than 0.998, and the structure was confirmed with ^{1}H NMR and ^{13}C NMR. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 8.85 (d, 1H, 3-H, $J = 2.0$ Hz), 8.38 (dd, 1H, 5-H, $J = 2.0$, 8.4 Hz), 7.63 (d, 1H, 6-H, $J = 8.4$ Hz), 3.99 (t, 1H, $-CH-$, $J = 7.6$ Hz), 3.48 (ddd, 2H, $-CH_2-$, $J = 7.2$, 8.0, 14.0 Hz). ^{13}C NMR (101 MHz, D_2O) δ: 170.23, 149.07, 148.29, 132.99, 131.80, 128.21, 122.60, 51.85, 35.77. Deionized water (with the electrical conductivity of $< 3 \cdot 10^{-6}$ S) was used in our experiment. Organic solvents (99.8 % in mass fraction) were purchased from Hangzhou Chemical Reagent Co., Ltd. of China and distilled before use. The purity of the 2,4-dinitro-L-phenylalanine was based on the ^{1}H NMR spectrum and HPLC.

Apparatus and Procedure. The solubility of a solid in a solvent was measured following the method reported in the literature.[6] In our previous work,[8] our co-worker had explained the procedure in detail, and little improvement was made. All the apparatuses including composition and type were exactly the same as with our previous work. So, here, we described it briefly. Our solubility apparatus included a 200 mL jacketed glass vessel, a thermostat, a magnetic stirrer, and a mercury-in-glass thermometer (uncertainty of $\pm$ 0.05 K). A laser beam was employed to observe the dissolution of the solid + liquid mixture. The light signal transmitted through the vessel was collected by a detector to guarantee the dissolution of the last crystal, and the equilibrium point of the given system was estimated on the basis of the signal change.

At the beginning of each experiment, a known mass of solute determined by an electronic analytical balance (type BS210S, Sartorius Scientific Instrument Co. Ltd.) with an uncertainty of less than 0.0001 g was added to a known mass of solvent at a known temperature. The undissolved solid particles were completely suspended in the jacketed vessel by continuous stirring for 60 min, and then a quantitative additional solvent was added into the vessel through a buret. The intensity of the penetrated light increased with the increase of the amount of solvent in the vessel, and the penetrated light intensity reached

10.1021/je100352r
Published on Web 08/10/2010

its maximum value when the last portion of the solid solute just disappeared. Then the mass of the solute and the total solvent were recorded. Together with the mass of the solute, the solubility would be obtained by the following equation

$$x_1 = \frac{m_1/M_1}{m_1/M_1 + m_2/M_2} \quad (1)$$

where m_1 and m_2 represent the mass of the solute and the solvent, and M_1 and M_2 denoted the molecular weight of the solute and the solvent, respectively.

All the experiments were repeated three times. The uncertainty of the experimental solubility values is about 0.5 %. The uncertainty in the solubility relative values is due to uncertainties in the temperature measurements, weighing procedure, and the stability of the water bath.

Results and Discussion

The solubilities of 2,4-dinitro-L-phenylalanine in the selected solvents were measured over the temperature range from (273.15 to 368.15) K and presented in Table 1, where T is the absolute temperatur and x_1 and x_1^{calcd} represent the solubility of the experimental and calculated values from the Apelblat equation, respectively. From Table 1, it can be seen that the solubility of 2,4-dinitro-L-phenylalanine in all selected solvents increases with the increase of temperature, and it is also shown that the solubility of 2,4-dinitro-L-phenylalanine is especially high in water, methanol, and 1-propanol but low in ethanol, 2-propanol, and 1-butanol and especially low in acetone, ethyl acetate, 2-butanol, and 1,2-dichloroethane. Furthermore, the solubility in 1-propanol and water varies much more obviously with temperature than in the other solvents, which may show its potential advantage in the recrystillization of 2,4-dinitro-L-phenylalanine. In addition, the solubility of 2,4-dinitro-L-phenylalanine seems rather "abnormal" compared to the regular amino acids. For example, its solubility behavior is very different in methanol and ethanol, which makes the measurement important for industrial application. The reason why it behaves so strangely might be that it contains two different types of groups: amino acid group−hydrophilic and 2,4-dinitrobenyl group−hydrophobic.

Amino acids were mainly in zwitterionic form in water[9] and in nonionic form in organic solvents, and it was suitable to employ the modified Apelblat equation to regression of the solubility data.[10] The solubility of 2,4-dinitro-L-phenylalanine as a function of temperature was fitted by the modified Apelblat equation[11]

$$\ln(x_1) = A + B/(T/\mathrm{K}) + C\ln(T/\mathrm{K}) \quad (2)$$

where A, B, and C are adjustable parameters and were obtained using the least-squares method and were presented in Table 2 together with the root-mean-square relative deviation (rmsrd), which is defined as

$$\mathrm{rmsrd} = \sqrt{\frac{\sum_{i=1}^{N}\left(\frac{x_i^{calcd} - x_i}{x_i}\right)^2}{N}} \quad (3)$$

where N is the number of experimental points.

Table 1. Solubility (x_1) of 2,4-Dinitro-L-phenylalanine (1) in Monosolvents from T = (273.15 to 368.15) K

T/K	x_1	$100(x_1 - x^{calcd})/x_1$	T/K	x_1	$100(x_1 - x^{calcd})/x_1$
water			ethanol		
273.15	$8.500\cdot10^{-8}$	0.53	273.15	$3.435\cdot10^{-5}$	−1.72
283.15	$1.121\cdot10^{-6}$	−0.61	283.15	$9.203\cdot10^{-5}$	1.20
293.15	$9.262\cdot10^{-6}$	−0.83	293.15	$1.953\cdot10^{-4}$	1.31
303.15	$5.124\cdot10^{-5}$	0.80	303.15	$3.500\cdot10^{-4}$	2.59
313.15	$1.892\cdot10^{-4}$	−0.93	313.15	$5.101\cdot10^{-4}$	−0.94
323.15	$5.232\cdot10^{-4}$	1.17	323.15	$6.710\cdot10^{-4}$	−0.67
333.15	$1.068\cdot10^{-3}$	2.02	333.15	$7.719\cdot10^{-4}$	−1.41
343.15	$1.660\cdot10^{-3}$	1.55	343.15	$7.902\cdot10^{-4}$	−2.80
353.15	$1.928\cdot10^{-3}$	−5.13	353.15	$7.913\cdot10^{-4}$	3.42
363.15	$1.983\cdot10^{-3}$	−3.17			
368.15	$2.006\cdot10^{-3}$	4.50			
methanol			acetone		
273.15	$1.497\cdot10^{-5}$	−2.02	273.15	$1.582\cdot10^{-6}$	−3.26
278.15	$4.123\cdot10^{-5}$	2.61	278.15	$1.645\cdot10^{-6}$	1.06
283.15	$9.103\cdot10^{-5}$	−2.27	283.15	$1.723\cdot10^{-6}$	1.12
288.15	$1.918\cdot10^{-4}$	−0.06	288.15	$1.911\cdot10^{-6}$	2.28
293.15	$3.648\cdot10^{-4}$	2.87	293.15	$2.148\cdot10^{-6}$	0.54
298.15	$5.780\cdot10^{-4}$	−2.12	298.15	$2.564\cdot10^{-6}$	0.79
303.15	$9.093\cdot10^{-4}$	1.83	303.15	$3.063\cdot10^{-6}$	−2.63
308.15	$1.234\cdot10^{-3}$	0.02	308.15	$3.989\cdot10^{-6}$	−0.83
313.15	$1.549\cdot10^{-3}$	−0.96	313.15	$5.257\cdot10^{-6}$	−1.11
318.15	$1.814\cdot10^{-3}$	−0.99	318.15	$7.138\cdot10^{-6}$	−1.43
323.15	$1.955\cdot10^{-3}$	−1.86	323.15	$1.010\cdot10^{-5}$	−0.47
328.15	$1.968\cdot10^{-3}$	−2.41	328.15	$1.452\cdot10^{-5}$	−0.46
333.15	$1.975\cdot10^{-3}$	3.26	333.15	$2.190\cdot10^{-5}$	1.79
ethyl acetate			1,2-dichloroethane		
303.15	$9.1\cdot10^{-9}$	−0.23	273.15	$2.2\cdot10^{-9}$	0.42
313.15	$1.88\cdot10^{-7}$	0.61	283.15	$8.6\cdot10^{-9}$	0.18
323.15	$1.878\cdot10^{-6}$	−3.10	293.15	$3.08\cdot10^{-8}$	−0.73
333.15	$1.123\cdot10^{-5}$	2.51	303.15	$1.051\cdot10^{-7}$	0.88
343.15	$3.578\cdot10^{-5}$	−0.97	313.15	$3.267\cdot10^{-7}$	−0.15
348.15	$5.437\cdot10^{-6}$	−0.45	323.15	$9.771\cdot10^{-7}$	1.06
			333.15	$2.691\cdot10^{-6}$	−0.36
2-propanol			1-propanol		
273.15	$1.511\cdot10^{-6}$	0.51	273.15	$2.581\cdot10^{-6}$	−0.13
278.15	$2.962\cdot10^{-6}$	0.03	283.15	$3.544\cdot10^{-6}$	0.68
283.15	$5.454\cdot10^{-6}$	−0.19	293.15	$5.642\cdot10^{-6}$	1.07
288.15	$9.491\cdot10^{-6}$	0.07	303.15	$1.006\cdot10^{-5}$	−0.31
293.15	$1.556\cdot10^{-5}$	0.10	313.15	$2.037\cdot10^{-5}$	−0.39
298.15	$2.422\cdot10^{-5}$	0.31	323.15	$4.546\cdot10^{-5}$	−0.57
303.15	$3.573\cdot10^{-5}$	0.21	333.15	$1.130\cdot10^{-4}$	1.49
308.15	$5.034\cdot10^{-5}$	0.26	343.15	$2.943\cdot10^{-4}$	0.85
313.15	$6.778\cdot10^{-5}$	0.25	353.15	$8.123\cdot10^{-4}$	−0.40
318.15	$8.751\cdot10^{-5}$	0.24	363.15	$2.413\cdot10^{-3}$	0.15
323.15	$1.086\cdot10^{-4}$	0.22	368.15	$4.235\cdot10^{-3}$	0.38
328.15	$1.296\cdot10^{-4}$	0.07			
333.15	$1.498\cdot10^{-4}$	0.17			
338.15	$1.672\cdot10^{-4}$	0.12			
2-butanol			1-butanol		
273.15	$6.490\cdot10^{-7}$	−4.18	273.15	$3.841\cdot10^{-6}$	−4.44
283.15	$6.790\cdot10^{-7}$	4.97	283.15	$3.909\cdot10^{-6}$	3.80
293.15	$6.920\cdot10^{-7}$	1.99	293.15	$3.994\cdot10^{-6}$	3.47
303.15	$7.790\cdot10^{-7}$	0.48	303.15	$4.281\cdot10^{-6}$	0.19
313.15	$9.450\cdot10^{-7}$	−0.89	313.15	$5.013\cdot10^{-6}$	−1.11
323.15	$1.233\cdot10^{-6}$	−1.35	323.15	$6.292\cdot10^{-6}$	−1.39
333.15	$1.711\cdot10^{-6}$	−1.21	333.15	$8.344\cdot10^{-6}$	−1.35
343.15	$2.492\cdot10^{-6}$	−1.12	343.15	$1.151\cdot10^{-6}$	−1.89
353.15	$3.871\cdot10^{-6}$	1.15	353.15	$1.687\cdot10^{-6}$	−0.36
363.15	$6.114\cdot10^{-6}$	1.34	363.15	$2.595\cdot10^{-6}$	2.48

It is seen that the calculated solubilities by the modified Apelblat equation are in good agreement with the experimental values.

Conclusions

The solubility of 2,4-dinitro-L-phenylalanine in all selected solvents is a function of temperature and increases with the rise of temperature.

The solubility of 2,4-dinitro-L-phenylalanine is especially high in water, methanol, and 1-propanol. The solubility in water and

Table 2. Parameters of the Modified Apelblat Equation for 2,4-Dinitro-L-phenylalanine in Different Solvents

solvent	*A*	*B*	*C*	100 rmsrd
water	1707.029	−89262.660	−248.934	2.474
methanol	1970.357	−94193.921	−288.445	2.036
ethanol	826.751	−40666.001	−119.379	1.189
acetone	−1002.783	41969.698	152.267	1.395
2-propanol	904.581	−46190.806	−130.207	0.230
1-propanol	−464.257	20152.985	70.672	2.471
2-butanol	−510.602	21905.614	77.472	2.340
1-butanol	−1023.244	42869.958	155.328	1.609
ethyl acetate	3463.687	−182895.419	−500.586	1.705
1,2-dichloroethane	−15.896	−8983.610	8.166	0.631

1-propanol varies much more obviously with temperature than in the other solvents.

The calculated solubility data by the modified Apelblat equation are in good agreement with the experimental values.

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Received for review April 12, 2010. Accepted July 23, 2010. We are grateful to the generous financial support from the National Natural Science Foundation of China (20776127), the National Key Technology R&D Program (2007BAI34B07), the Natural Science Foundation of the Zhejiang Province (Y4090045, R4090358), and the Research Foundation of Education Bureau of Zhejiang Province under Grant No. Y200908295.

JE100352R