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The aqueous solubility and thermal behaviour of some β -carbolines

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Abstract

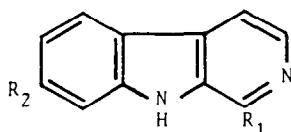
The solubilities of the three β -carbolines, harmine, harmine and norharmine, in water at pH 13 have been determined spectrophotometrically over the temperature range 288–318 K. Plots of logarithms of solubility are linear functions of reciprocal temperature, and enthalpies of solution have been determined from these. For comparison, the melting of these compounds has been studied by DSC. All show a single sharp endothermic transition. However, the enthalpies of fusion are greater than the enthalpies of solution determined from solubility data, with the biggest difference occurring for harmine. In part, this may be a result of the thermal decomposition of this compound contributing to the observed transition enthalpy. It is also suggested that acid–base equilibria significantly affect the solubility.

Keywords: Aqueous solubility; β -Carbolines; Melting transition

1. Introduction

The simple β -carboline alkaloids are a group of naturally occurring compounds which possess a common tricyclic pyrido(3,4-b)indole ring structure. They are widely distributed in various plant systems [1], and, in addition, have been detected in tobacco smoke [2], certain roasted foodstuffs [3], mammalian tissues [4], and as photodeg-

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(1) NORHARMANE	R ₁ =H	R ₂ =H
(2) HARMANE	R ₁ =CH ₃	R ₂ =H
(3) HARMINE	R ₁ =CH ₃	R ₂ =OCH ₃

Fig. 1. Structures of the β -carboline.

radation products of human lenses [5] and wool [6]. In the majority of cases, these systems are believed to be derived from reactions of tryptophan. The β -carboline show considerable pharmacological activity [7], and recently interest has been shown in the application of their phototoxicity towards bacteria, fungi, viruses and insects in the area of pest control [8]. However, in spite of the importance of quantitative data on the solubility of these molecules in the interpretation of their behaviour in biological systems, little information exists on this in the literature. Qualitative measurements have been made on the polarity of the molecules using chromatography [9,10], and distribution coefficients between 1-octanol and water have been reported [10]. We have been studying the photophysical behaviour of certain β -carboline [11,12] to help understand aspects of the mechanism of their photosensitization *in vivo*. In connection with this work, we have measured the solubilities of the three β -carboline, harmine (1), harmane (2) and norharmane (3) (Fig. 1), in aqueous solutions at various temperatures. To facilitate understanding of this data, we have also studied their phase transitions using differential scanning calorimetry (DSC).

2. Experimental

2.1. Materials

Harmine, harmane and norharmane (Aldrich) were normally used without further treatment. Purity was confirmed by DSC. Harmine and norharmane were also recrystallized from water for some of the measurements. However, no significant differences were observed between the behaviour of these samples and the unrecrystallized products. All solubility measurements were carried out in doubly distilled water.

2.2. Apparatus and methods

Differential scanning calorimetry measurements were carried out on 3–4 mg samples with a Polymer Laboratories PL-DSC apparatus in a nitrogen atmosphere using heating rates of $10^{\circ}\text{C min}^{-1}$. Visual observations of melting were made on an H. Bock

Monoscope optical hot stage. The solubilities of harmine, harmane and norharmane were measured at pH 7 and 13, and at various temperatures from 15 to 45°C using the absorbance at the maxima in the spectra of saturated solutions and the respective molar extinction coefficients. Generally, measurements were made at two of the absorption bands of the compounds. Molar extinction coefficients were determined experimentally, giving values of: harmine, $\log \epsilon_{246\text{nm}} = 4.53$, $\log \epsilon_{319\text{nm}} = 4.20$; harmane, $\log \epsilon_{299\text{nm}} = 4.08$; $\log \epsilon_{365\text{nm}} = 3.57$; norharmane, $\log \epsilon_{301\text{nm}} = 4.03$, which are in reasonable agreement with literature data [13–15]. For the solubility measurements, saturated solutions were prepared at temperatures above the values of interest, and were then allowed to equilibrate at the temperature of measurement. Spectra were measured every twelve hours to study the approach to equilibrium, and the final measurements were made after no further change in absorbance was observed (normally 48 h.). Solubilities were obtained as molarities, and converted to molalities and mole fractions using literature data for the density of water at the respective temperature [16].

3. Results and discussion

Harmine, harmane and norharmane were studied by DSC on heating over the temperature range 25–300°C. In all cases, only a single sharp endothermic peak is observed (Fig. 2). Visual observations on a hot stage confirm that this is the melting transition. In agreement with previous reports [17], with harmine, the studies on the hot stage show that this compound decomposes on melting to form a yellow-brown residue. Melting temperatures, enthalpies and entropies of fusion are given in Table 1. In general, the transition temperatures are in good agreement with literature values for the melting points [16–18]. The biggest disagreement is with one literature value for harmine [16]. However, the agreement between our value and other literature data [17,18] suggests that the value in Ref. [16] may be wrong.

The solubilities of the three β -carbolines were determined at pH 7 and 13 at 20°C. Preliminary results have already been reported [12], and show that the solubilities increase on decreasing pH. However, this can be readily understood, as the β -carbolines all protonate in the pH range 7–8 [13–15], and the enhanced solubility is a reflection of the increasing fraction of the more soluble cationic form on decreasing pH. For all subsequent measurements, solubilities were determined at pH 13, where only the neutral form of the compounds is expected to be present. Solubilities have been determined at various temperatures over the range 15–45°C, and are presented in Table 2. The order of solubilities at room temperature (harmine < harmane < norharmane) closely follows the order of decreasing enthalpy of fusion, as expected for ideal behaviour [19]. The logarithms of the solubility are good linear functions of reciprocal temperature, as seen in Fig. 3.

The solubilities in this case are determined as mole fractions, and the appropriate relationships for these systems are

$$\ln \chi_m = -a/T + b$$

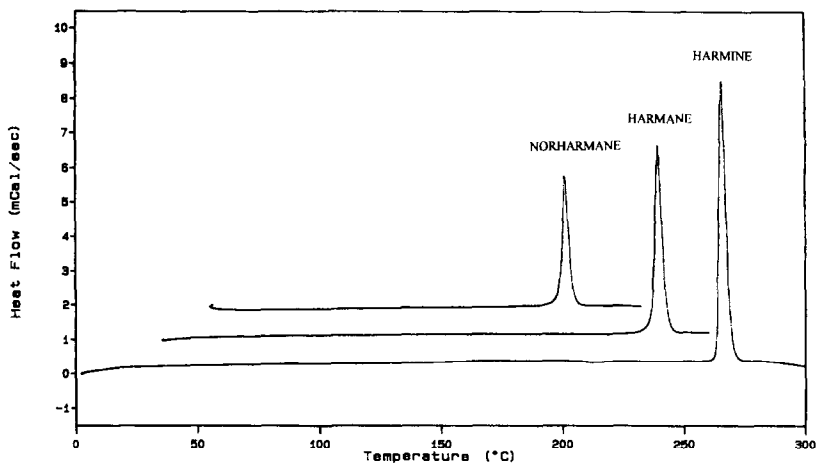


Fig. 2. DSC traces observed upon heating norharmine, harmane and harmine. Heating rate, $10^{\circ}\text{C min}^{-1}$.

Table 1
Thermodynamic data obtained for harmine, harmane and norharmine

Compound	Fusion melting point/K		$\Delta H_f/\text{kJ mol}^{-1}$	$\Delta S_f/\text{J K}^{-1}\text{mol}^{-1}$	Solution $\Delta H_{\text{sol}}/\text{kJ mol}^{-1}$
	This work ^a	Lit. value			
Harmine	536.6 (538.6)	534 ^b , 530–2 ^c , 545–7 ^d	48.8	91.0	11.9
Harmane	509.9 (512.2)	508–511 ^{b,c,d}	27.2	53.4	25.5
Norharmine	471.5 (473.8)	471.5 ^c	25.5	50.0	18.5

^a Determined from onset of the melting transition. For convenience, values of the maxima are also given in brackets.

^b From Ref. [17].

^c From Ref. [18].

^d From Ref. [16].

with $a = 1430 \text{ K}^{-1}$, $b = -3.005$, $r(\text{regression coefficient}) = 0.9956$ (harmine); $a = 3066 \text{ K}^{-1}$, $b = +3.864$, $r = 0.9910$ (harmane); $a = 2229 \text{ K}^{-1}$, $b = +1.781$, $r = 0.9914$ (norharmine). From these graphs the enthalpies of solution have been determined, as described by Williamson [20] (Table 1). As indicated earlier, these should be related to the enthalpies of fusion [19]. It should be noted that these are not true thermodynamic enthalpies, as under the conditions used (ionic strength 0.1 M) correction needs to be made for using concentration instead of activity of the β -carbolines. However, the systems can be treated as ones at constant ionic strength, such that direct comparison of solubility and melting data is valid. From this table, qualitative agreement is

Table 2

Solubilities of harmine, harmane and norharmine in aqueous solution at pH 13 at various temperatures

Temperature/K	Solubility/ 10^{-5} mol kg $^{-1}$		
	Harmine	Harmane	Norharmine
288	1.88	6.01	–
289	1.94	6.25	13.91
290	2.00	6.71	16.01
293	–	8.36	–
310	2.81	13.64	25.35
311	2.75	14.34	25.61
318	2.98	16.17	29.16

observed between the enthalpies of solution and fusion of harmane and norharmine, but the value obtained for fusion of harmine is four times that obtained from solubility from Fig. 3. The observation of thermal decomposition of this compound on melting suggests that this process may contribute to the observed value for ΔH_{fus} . However, both the sharp DSC trace observed for this system in Fig. 2 and the qualitative agreement between the fusion enthalpies and the order of solubilities at room temperature are difficult to explain in terms of a major contribution of thermal decomposition to this DSC peak. An alternative explanation is that some other term is contributing significantly to the ΔH_{sol} value. It is known [15] that these three compounds do ionize at $\text{pH} > 14$, and it is possible that such acid–base equilibria may contribute to the observed non-ideal behaviour.

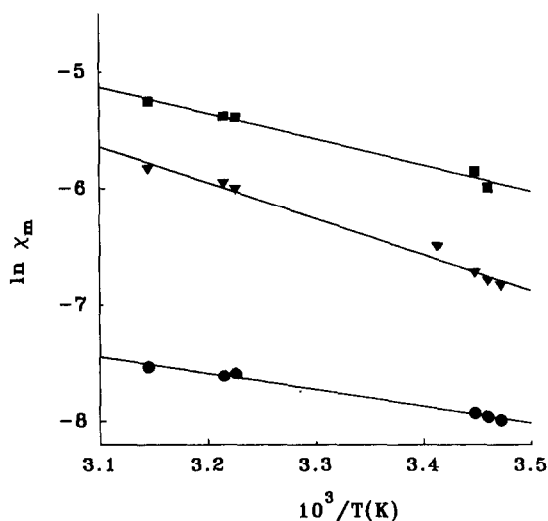


Fig. 3. Logarithmic plots of solubility against reciprocal temperature for norharmine (■), harmane (▲) and harmine (●).

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