

## CHEMICAL THERMODYNAMICS AND THERMOCHEMISTRY

# Thermochemistry of Flavonoid Myricetin

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**Abstract**—The enthalpies of myricetin dissolution are measured by means of calorimetry with mol dilutions of flavonoid : 96 mol % ethanol equal to 1 : 9000, 1 : 18 000 and 1 : 36 000. The standard enthalpies of dissolution for the biologically active substance in an infinitely diluted (standard) solution of 96% ethanol are calculated from the experimental data. Physicochemical means of approximation are used to estimate the values of the standard enthalpy of combustion, and the enthalpy of melting is calculated for the investigated flavonoid. Finally, the compound's standard enthalpy of formation is calculated using the Hess cycle.

**Keywords:** flavonoid myricetin, thermodynamic characteristic, calorimetry.

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## INTRODUCTION

Flavonoids are most common polyphenolic compounds of vegetable origin. More than 4000 different flavonoids are currently known. The pharmacological effects of flavonoids were discovered in 1936 by Szent-Gyorgyi, who identified the vitamins of the P group and determined their polyphenol structure [1, 2]. The diverse biological activities of flavonoids is due to the presence of reactive hydroxyl and carbonyl groups in their molecules. These compounds are also capable of transforming into various quinones in biological systems. However, most flavonoids are found in plants and enter the human body in the form of glycosides. Molecules of flavonoid can be also acetylated and methylated.

Fundamental and applied research into natural polyphenols served as the basis for the creation of a whole area of the pharmaceutical industry that produces hundreds of types of medicines, food, and biologically active additives (BAA) whose main component is flavonoids. One feature of the biological action of flavonoids is the extremely wide range of potential targets on which they can act in the body, attracting the interest of chemists and physical chemists [3, 4]. Calorimetry is of particular interest in the standardization of drugs [5]. In this work, we present the results from thermochemical and thermodynamic studies of the biologically active substance flavonoid myricetin. We could find no such data in the literature.

## EXPERIMENTAL

Our biologically active compound C<sub>15</sub>H<sub>10</sub>O<sub>8</sub> was obtained from roots of the limonium plant at the Faculty of Chemistry, Kozybayev North Kazakhstan State University using the technique described in [6].

The first stage was extraction by ethanol. The crushed roots of limonium were percolated with 96% ethanol at 25°C for 3 days. The resulting extract was then concentrated to half its initial volume at 50°C under vacuum. The second step was the hydrolysis of glycosides. The resulting concentrated extract was hydrolyzed with 4% sulfuric acid in a volume ratio of 1 : 1 for 2 hours with heating to 95–100°C until a yellow crystalline precipitate that was a mixture of flavonoid aglycones appeared. The third step was the purification of aglycones. The resulting precipitate was filtered off after hydrolysis and washed thoroughly with distilled water to remove phenol acids and the by-products of hydrolysis. The washed precipitate (the mixture of flavonoid aglycones) was dried at 100°C for two hours. Dry substance was extracted three times with diethyl ether to purify it of resins and related myricetin flavonols and was dried in air and then in an oven for two hours at a temperature of 160°C. After recrystallization from 70% aqueous ethanol, myricetin with a melting point of 45–347°C was obtained:  $R_f = 0.64$  in the solvent system n-butanol–acetic acid–water (4 : 1 : 5); for the UV spectrum in C<sub>2</sub>H<sub>5</sub>OH,  $\lambda_{\max} = 380$  and 255 nm. The yield was 2% of the weight of the dry roots.

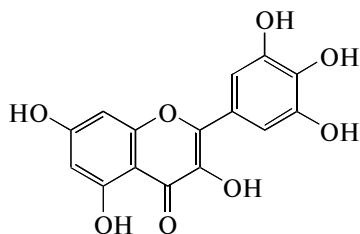
The resulting myricetin was analyzed by means of high-pressure reverse-phase high performance liquid chromatography (HPLC) on a Hewlett–Packard Agi-

Enthalpy of dissolution of myricetin in 96% ethanol at dilutions (mol of  $C_{15}H_{10}O_8$  : mol of 96% ethanol) of 1 : 9000, 1 : 18 000 and 1 : 36 000

| Number     | Mass, g | $\Delta H_{\text{dissol}}$ , J | $\Delta H_{\text{dissol}}^m$ , kJ/mol |
|------------|---------|--------------------------------|---------------------------------------|
| 1 : 9000   |         |                                |                                       |
| 1          | 0.0030  | 0.2086                         | 22.13                                 |
| 2          | 0.0031  | 0.2188                         | 22.46                                 |
| 3          | 0.0032  | 0.2221                         | 22.06                                 |
| 4          | 0.0032  | 0.2241                         | 22.29                                 |
| 5          | 0.0031  | 0.2156                         | 22.13                                 |
| average    |         |                                | $22.22 \pm 0.72$                      |
| 1 : 18 000 |         |                                |                                       |
| 1          | 0.0018  | 0.0978                         | 17.29                                 |
| 2          | 0.0018  | 0.0981                         | 17.34                                 |
| 3          | 0.0017  | 0.0950                         | 17.78                                 |
| 4          | 0.0015  | 0.0816                         | 17.32                                 |
| 5          | 0.0015  | 0.0803                         | 17.04                                 |
| average    |         |                                | $17.35 \pm 1.02$                      |
| 1 : 36 000 |         |                                |                                       |
| 1          | 0.0008  | 0.0350                         | 13.92                                 |
| 2          | 0.0008  | 0.0340                         | 13.52                                 |
| 3          | 0.0009  | 0.0380                         | 13.44                                 |
| 4          | 0.0008  | 0.0340                         | 13.52                                 |
| 5          | 0.0009  | 0.0390                         | 13.79                                 |
| average    |         |                                | $13.64 \pm 1.71$                      |

lent 1100 Series instrument. The analytical column was packed with Zorbax SB-C<sub>18</sub> sorbent (dimensions,  $4.6 \times 150$  mm; particle size, 5  $\mu\text{m}$ ; mobile phase, methanol and 5% solution of acetic acid in water at a ratio of 40 : 60; detection at a wavelength of 254 nm; column at room temperature; mobile phase rate, 0.5 ml/min; amount of sample injected, 20  $\mu\text{L}$ ). The data were processed using the ChemStation software.

The results from our analysis showed that the purity of myricetin was  $99.22 \pm 0.05\%$ . The structural formula of myricetin is shown below.



The enthalpy of dissolution for myricetin in 96% ethanol was investigated using a DAC-I-IA calorimeter at 25°C. The theory and technique of calorimetric study were described in detail in [7–9]. The calorimeter's range of operating temperatures was 25–200°C;

the volume of the ampoules was 8 cm<sup>3</sup>; the length of operation was up to 5 hours; the sensitivity of the direct measurement of thermal EMFs was no lower than 0.12  $\mu\text{V}/\mu\text{W}$ ; the sensitivity in the regime of automatic compensation for thermal EMFs was at least 0.012  $\mu\text{V}/\mu\text{W}$ ; and the error in measuring thermal power did not exceed 2.0%. Thermal effects were registered using a KSP-4 potentiometer and an IP-4 precision integrator in parallel. The period of preliminary thermostating was 2 h.

The instrument's operation was confirmed by measuring the  $\Delta H^\circ$  of dissolution of thrice recrystallized KCl at dilutions of 1 : 1600, 1 : 2400 and 1 : 3200 (mol of salt : mol of water). The value of  $\Delta H^\circ$  was  $17860 \pm 283$  kJ/mol, in good agreement with its recommended values [10, 11].

Experimental determination of the enthalpy of dissolution of myricetin in 96% ethanol was performed at dilutions of 1 : 9000, 1 : 18 000 and 1 : 36 000 (mol of compound : 96 mol % ethanol). The results from calorimetric determination of the enthalpy of dissolution of myricetin at different dilutions are shown in the table. The errors of parallel experiments and the homogeneity of their variances were calculated using the tests of Student and Cochran [12]. It should be noted that the error in measuring the enthalpy of dissolution at different dilutions was within the DAK-I-IA calorimeter's accuracy of determining thermal effects (~2.0%): 0.86% at a dilution of 1 : 9000; 1.9% at 1 : 18 000; and 1.9% at 1 : 36 000. Since weighing was performed with a precision of one unit in the last digit, the accuracy was 3.23% at a dilution of 1 : 9000; 5.88% at a dilution of 1 : 18 000; and 12.51% at 1 : 36 000 (see table).

## RESULTS AND DISCUSSION

Our experimentally determined values for the enthalpy of dissolution of  $C_{15}H_{10}O_8$  at different dilutions were used to calculate the enthalpy of dissolution at infinite (standard) dilution. Mathematical processing of the experimental data revealed that as in [13], the dependence of enthalpy of dissolution  $\Delta H_{\text{dissol}}^m$  of the compound on molal concentration  $\sqrt{m}$  was described by the equation

$$\Delta H_{\text{dissol}}^m(C_{15}H_{10}O_8) = 5.60 + 339.14\sqrt{m}, \quad (1)$$

the solving of which revealed that the standard enthalpy of solution for myricetin in a standard (infinitely dilute) solution of 96% ethanol was  $5.60 \pm 1.15$  kJ/mol. It should be noted that myricetin is a molecular compound; we therefore assumed its complete dissolution in a solution of 96% ethanol at the above dilutions when applying Eq. (1) to myricetin.

The Harash equation used to calculate  $\Delta H^\circ$  of combustion had the form

$$\Delta H_{\text{combust}}^\circ(298.15), \text{ kcal/mol} = 26.050(4n_{\text{C}} + n_{\text{H}} - p) + \sum k_i \Delta_i, \quad (2)$$

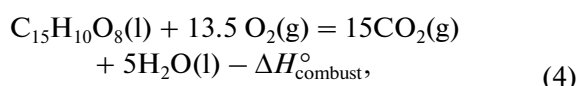
where 26.050 kcal/mole was the heat of C–C, C–H bond breaking and the subsequent formation of  $\text{CO}_2$  and  $\text{H}_2\text{O}$ ;  $n_{\text{C}}$  is a number of carbon atoms in the compound;  $n_{\text{H}}$  is the number of hydrogen atoms in the compound;  $p$  is the number of partially shifted electrons in a molecule of compound;  $k_i$  is the number of identical substituents; and  $\Delta_i$  is a thermal adjustment that corresponds to a given substituent. The values of  $\Delta_i$  were presented in tabular form in [14].

Frost improved on Harash's method. The value of  $\Delta H_{\text{combust}}^\circ(298.15)$  according to Frost was calculated as

$$\Delta H_{\text{combust}}^\circ(298.15), \text{ kcal/mol} = -(104.2n_{\text{C}} + 26.05n_{\text{H}} + 13.0n_{\text{=}} + 46.1n_{\text{=}} + 6.5n_{\text{Cy}} - 3.5n_{\text{Ar-Alk}} - 6.5n_{\text{Ar-Ar}}), \quad (3)$$

where  $n_{\text{C}}$  is the number of carbon atoms in the molecule;  $n_{\text{H}}$  is the number of hydrogen atoms;  $n_{\text{=}}$  is the number of double bonds in a molecule of alkene or in the side chains of cyclic compounds;  $n_{\text{=}}$  is the number of triple bonds in alkyne molecules;  $n_{\text{Cy}}$  is the number of double bonds in the ring cyclenes;  $n_{\text{Ar-Alk}}$  is the number of bonds between aryl and alkyl groups; and  $n_{\text{Ar-Ar}}$  is the number of bonds between aryl groups [14]. It should be noted that the Harash and Frost techniques are approximate methods for calculating the enthalpy of combustion.

For myricetin, the average value of  $\Delta H_{\text{combust}}^\circ(298.15)$  calculated using Eqs. (2) and (3) was 10 kJ/mol. Using the average value of  $\Delta H_{\text{combust}}^\circ(298.15)$  according to the Hess cycle, the standard enthalpy of formation of this compound in the liquid state was calculated from the reaction



$238.8 \pm 10.0$  kJ/mol.

To calculate  $\Delta_f H^\circ(298.15 \text{ K})$   $\Delta_f H^\circ(\text{CO}_2(\text{g}), 298.15 \text{ K}) = (-393.51 \pm 0.05 \text{ kJ/mol})$  and  $\Delta_f H^\circ(\text{H}_2\text{O}(\text{l}), 298.15 \text{ K}) = (-285.83 \pm 0.04 \text{ kJ/mol})$  were taken from [15, 16].

Since myricetin is in the crystalline state at the standard temperature of 298.15 K, its  $\Delta_f H^\circ(298.15 \text{ K})$  in the solid state must be calculated. This requires knowledge of the  $\Delta_f H^\circ$  of melting. Gambill [17] found empirically that the entropy of melting for organic compounds (containing hydrogen, carbon, oxygen) can be approximated using the formula

$$\Delta S_{\text{melt}}^\circ = 20.72 \times 10^{0.00324M}, \quad (5)$$

where  $M$  is the molecular mass of the compound. For  $\Delta H_{\text{melt}}^\circ$ , the equation can be written as

$$\Delta H_{\text{melt}}^\circ / T_{\text{melt}} = 20.72 \times 10^{0.00324M}. \quad (6)$$

Considering the average melting point of myricetin ( $346^\circ\text{C}$  (619 K)), which is consistent with the data in

[5], and its molecular weight (318.2392),  $\Delta H_{\text{melt, flavonI}}^\circ$  was found to be 137.8 kJ/mol. As in [17], the accuracy of the method for calculating  $\Delta H_{\text{melt}}^\circ$  was not specified; we took it to be  $\sim 5.0\%$ , since this error is allowed in many approximate methods of calculation. Based on the above, the value of  $\Delta H_{\text{melt}}^\circ$  for myricetin was found to be  $137.8 \pm 6.9$  kJ/mol. Using the equation

$$\Delta_f H^\circ(\text{C}_{15}\text{H}_{10}\text{O}_8(\text{s}), 298.15 \text{ K}) = \Delta_f H^\circ(\text{C}_{15}\text{H}_{10}\text{O}_8(\text{l}), 298.15 \text{ K}) - \Delta H_{\text{melt}}^\circ \quad (7)$$

the standard enthalpy of formation of  $\text{C}_{15}\text{H}_{10}\text{O}_8$  in the crystalline state was calculated to be  $-376.6 \pm 12.1$  kJ/mol.

## CONCLUSIONS

The standard enthalpies of dissolution in an infinitely diluted (standard) solution of 96% ethanol myricetin ( $5.60 \pm 1.15$  kJ/mol) were determined for the first time by means of calorimetry via the extrapolation of experimental data at different dilutions. The enthalpy of combustion for myricetin  $\text{C}_{15}\text{H}_{10}\text{O}_8$  ( $-7093.5 \pm 10$  kJ/mol) was estimated using approximate methods, and the enthalpy of melting for the compound was found to be  $137.8 \pm 6.9$  kJ/mol. Its standard enthalpies of formation in the liquid and crystalline states was calculated to be  $-238.8 \pm 10.0$  and  $-376.6 \pm 12.1$  kJ/mol, respectively. The resulting thermodynamic and thermochemical characteristics are of both theoretical interest and practical value in studying the energy patterns of processes that occur with the participation of biologically active substances.

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