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HYDROPHOBICITY CONSTANTS FOR SEVERAL XANTHONES AND FLAVONES

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The octanol–water distribution of several xanthones and flavones was studied. Their hydrophobicity constants (log P) were determined. The lipophilicity constants (π) of the substituents were calculated. The relationship between the structure and hydrophobicity constants of the gamma-pyrone derivatives was found.

Keywords: xanthones, flavones, hydrophobicity constants, substituent lipophilicity constants.

Xanthones and flavones are secondary plant metabolites and exhibit broad spectra of biological activity [1–3]. Therefore, the study of the physicochemical properties of these gamma-pyrone derivatives is attractive [4–6]. An important physicochemical property of biologically active compounds is the hydrophobicity constant, which determines their ability to penetrate cell membranes. Knowledge of this constant is a reliable predictor of the media in which the compound will accumulate in vivo, i.e., in lipids of cell membranes or in aqueous media.

The hydrophobic properties of gamma-pyrone derivatives were not systematically studied before our research [7]. We previously determined the hydrophobicity constants of flavone and several of its monosubstituted derivatives [8]. Herein we present results from a study of the hydrophobic properties of several xanthones and disubstituted flavones.

Flavones and xanthones that are isolated from plants are, as a rule, polysubstituted compounds. Therefore, we used simpler synthetic mono-, dihydroxy-, and methoxy-substituted analogs in additional to natural compounds and their modified derivatives in the study. This set of samples was studied because their biological activity is determined by differences in the number and location of the hydroxyls and methoxyls in the molecules [1, 3, 9].

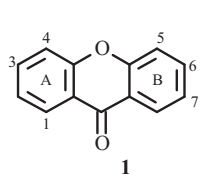
The octanol–water distribution of the compounds was studied. This system was selected because octanol mimics the cell-membrane lipid layer. Therefore, the hydrophobicity constants obtained in this system can be used for structure–activity correlations [10, 11]. The distribution coefficients were calculated using the formula

$$P = C_o/C_w,$$

where C_o and C_w are the equilibrium molar concentrations of the compounds in the organic and aqueous phases [12].

The concentrations of the compounds after equilibration were determined in each phase using UV spectroscopy. For this, plots of optical density vs. concentration at the analytical wavelengths were constructed beforehand. Figure 1 shows an example of UV spectra for three xanthones with different substituents.

The wavelengths (λ) at which the absorption was maximal were used as the analytical ones for xanthones with strong absorption in the range 250–265 nm: xanthone (**1**), 265 nm; 1-hydroxyxanthone (**3**), 250; 4-methoxyxanthone (**5**), 248; 1,7-dihydroxy-3,8-dimethoxyxanthone (**7**), 260; 1-hydroxy-2,3,4,5-tetramethoxyxanthone (**8**), 265; and 1-allyloxy-2,3,4,5-tetramethoxyxanthone (**9**), 250.



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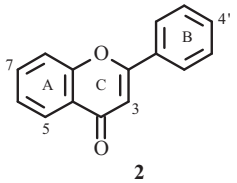

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TABLE 1. Octanol–Water Hydrophobicity Constants (log P) of Several Gamma-Pyrone Derivatives; Substituent Lipophilicity Constants (π)

Compound	log P	Substituent	π
Xanthone (**1**)	3.20 ± 0.05		
1-Hydroxyxanthone (**3**)	3.70 ± 0.06	1-OH	0.50
3-Hydroxyxanthone (**4**)	2.86 ± 0.07	3-OH	–0.34
4-Methoxyxanthone (**5**)	3.05 ± 0.05	4-OCH_3	–0.15
1,3-Dihydroxyxanthone (**6**)	3.77 ± 0.05	1-OH	0.91
		3-OH	0.07
1,7-Dihydroxy-3,8-dimethoxyxanthone (**7**)	3.50 ± 0.09	–	–
1-Hydroxy-2,3,4,5-tetramethoxyxanthone (**8**)	3.40 ± 0.06	1-OH	–0.30
		2,3,4,5-OCH_3	
1-Allyloxy-2,3,4,5-tetramethoxyxanthone (**9**)	3.60 ± 0.07	1-CH_2-CH=CH_2	0.20
Flavone (**2**)*	3.47 ± 0.05	–	–
7-Hydroxyflavone (**10**)*	3.56 ± 0.07	7-OH	0.09
5-Hydroxyflavone (**11**)*	3.77 ± 0.09	5-OH	0.30
3-Hydroxyflavone (**12**)*	4.17 ± 0.05	3-OH	0.70
4′-Hydroxyflavone (**13**)*	3.30 ± 0.05	4′-OH	–0.17
4′-Methoxyflavone (**14**)*	3.52 ± 0.05	4′-OCH_3	0.05
3-Methoxyflavone (**15**)*	3.58 ± 0.07	3-OCH_3	0.11
5,7-Dihydroxyflavone (**16**)	3.90 ± 0.05	7-OH	0.13
		5-OH	0.34
5,4′-Dihydroxyflavone (**17**)	4.10 ± 0.1	4′-OH	0.33
		5-OH	0.80
3-Hydroxy-7-methoxyflavone (**18**)	3.90 ± 0.08	3-OH	–
		7-OCH_3	–0.27

*Hydrophobicity constants of **2** and **10**–**15** were determined earlier [8] and were used to compare the relationship between structure and hydrophobic properties of several flavones and xanthones.

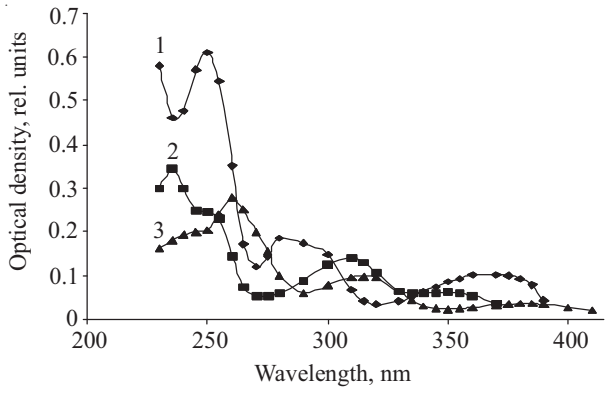

Fig.1

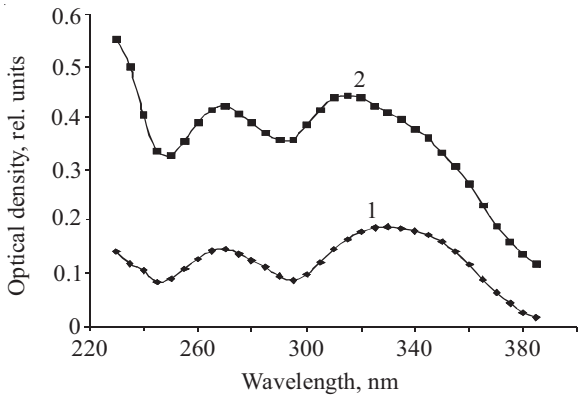

Fig. 2

Fig. 1. UV spectra of xanthones in aqueous MeOH (10%): 1-hydroxyxanthone ($C = 2.5 \cdot 10^{-5}$ M, $l = 1$ cm) (1), 1,3-dihydroxyxanthone ($C = 1.2 \cdot 10^{-5}$ M, $l = 1$ cm) (2), 1-hydroxy-2,3,4,5-tetramethoxyxanthone ($C = 2 \cdot 10^{-5}$ M, $l = 1$ cm) (3). Fig. 2. UV spectrum of 5,4′-dihydroxyflavone in aqueous MeOH (10%): $C = 1 \cdot 10^{-5}$ M, $l = 1$ cm (1); $C = 5 \cdot 10^{-5}$ M, $l = 1$ cm (2).

UV spectra of 3-hydroxyxanthones (Fig. 1) had their strongest absorption bands in the range 220–240 nm. Because octanol absorbs strongly in this spectral range, long-wavelength absorption maxima for 3-hydroxyxanthone (**4**) (305 nm) and 1,3-dihydroxyxanthone (**6**) (250 nm) were used as the analytical ones. Calibration curves for 5,7- and 5,4′-dihydroxyflavones were constructed at 270 nm; for 3-hydroxy-7-methoxyflavone, at 320.

The main difficulty in determining the distribution coefficients of the xanthones and flavones was their high hydrophobicity. Aqueous solutions containing MeOH (10%) with compound concentrations 10^{-6}–10^{-5} M were used to construct the plots of optical density (D) vs. concentration (C) because of the poor solubility of the compounds in water. A deviation

from Beer's law ($D = \varepsilon \cdot C \cdot l$) was observed on the calibration curves of 5,7- and 5,4′-dihydroxyflavones (**16** and **17**) at concentrations greater than $2.5 \cdot 10^{-5}$ M [13].

The high hydrophobicity of these compounds probably facilitated the formation of associates in aqueous medium. For example, a shift of the long-wavelength maximum from 330 to 315 nm and a change of intensity of the long-wavelength and short-wavelength (λ 270 nm) absorption bands as the concentration of 5,4′-dihydroxyflavone (**17**) increased was consistent with this (Fig. 2).

In order to exclude the influence of association on the distribution coefficients, experiments were conducted with a large octanol—water phase ratio (1:40, 1:50, 1:60) and the maximum initial compound concentration in octanol (10^{-2} M).

Table 1 presents the results.

The hydrophobicity constant is known to depend on the number of C atoms in the molecule [12]. Unsubstituted xanthone (**1**) contains one C atom less than flavone (**2**). Therefore, flavones are more hydrophobic. The log P values for xanthones lie in the range 2.90–3.80; for flavones, 3.30–4.20.

In order to assess quantitatively the influence of the substituents on log P of flavones and xanthones, we calculated in all instances where possible the substituent lipophilicity constants (π):

$$\pi = \log P_x - \log P,$$

where P and P_x are the distribution constants of the compound and its substituted derivative and π, the substituent lipophilicity constant [12].

We showed earlier [8] that a hydroxyl in the 4′-position decreases the hydrophobicity of flavone (**2**). This same group in the 7-, 5-, and 3-positions increases it more the closer it is to the electron-accepting carbonyl. The hydrophilic nature of the OH group depends on its ability to form solvates and hydrates. The carbonyl withdraws electron density from the oxygen and decreases the ability of the OH group to form a H-bond with water. In contrast with flavones, introducing a hydroxyl in the position *para* (3-) to the carbonyl reduces the hydrophobicity of 3-hydroxyxanthone (**4**).

A hydroxyl substituent in the *peri*-position to the carbonyl increases the hydrophobicity of xanthone ($\pi_{1\text{-OH}} = +0.47$) and flavone ($\pi_{5\text{-OH}} = +0.30$). Formation of an intramolecular H-bond (IHB), which hinders solvation, may cause an increase of the hydrophobicity in 5-hydroxyflavone (**11**) and 1-hydroxyxanthone (**3**). The influence of the substituent in the *peri*-position on the hydrophobicity of flavones and xanthones becomes definitive with two hydroxyls in ring A.

Table 1 shows that introducing a second hydroxyl into **11** increases the hydrophobicity. However, whereas the substituent hydrophobicity constants in 5,7-dihydroxyflavone (**16**) change little compared with those of 5-hydroxy- and 7-hydroxyflavones, both constants are significantly greater in 5,4′-dihydyroxyflavone (**17**) than in the corresponding monosubstituted derivatives. The hydroxyl in the 5-position in **17** (pK 12.72 [6]) is bonded to the carbonyl through a strong IHB that hinders its solvation. Therefore, it has a high π-constant.

The combined effect of two hydroxyls in 1,3-dihydroxyxanthone (**6**) increases the hydrophobicity constant. Both π_{OH} constants increase compared with those of the monosubstituted derivatives.

The value of the hydroxyl π-constant in flavones and xanthones depends on its position in the molecules and exceeds significantly the value of the constant for a phenol hydroxyl [$\pi_{OH} = -0.67$]. This is indicative of the definitive influence of electronic interactions between the carbonyl and hydroxyl substituents on the hydrophobicity of gamma-pyrone derivatives.

Methoxyls in the 3- and 4′-positions of flavone (**15** and **14**) increase slightly its hydrophobicity. However, the hydrophobicity constant of 3-hydroxyflavone decreases with a methoxyl in the 7-position (**18**, Table 1).

Introducing a methoxyl into the 4-position of xanthone also decreases its hydrophobicity constant, i.e., increases its hydrophilicity ($\pi_{OCH_3} = -0.15$). Natural xanthones contain very often methoxyl substituents that increase their hydrophilicity. For example, the hydrophobicity constant of 1,7-dihydroxy-3,8-dimethoxyxanthone (**7**) was less than that of 1,3-dihydroxyxanthone (**6**). Increasing the number of methoxyls more reduced even more the hydrophobicity constant (**8**, Table 1).

Allylation of **8** produced 1-allyloxyxanthone **9** and increased the hydrophobicity, as expected [12].

It is much more complicated to assess the influence of each group on the hydrophobicity in polysubstituted xanthones because of the lack of simple analogs that could be used to calculate the π-constant in each particular instance.

Experimental determination of the distribution coefficients is exceedingly labor-intensive. Therefore, studies in which hydrophobicity constants were determined by calculations have recently appeared [10, 11]. However, they give good results only for simple molecules. The accuracy of the calculated lipophilicity of compounds with several functional groups is still not high enough. Table 1 shows that the hydrophobicity constants of flavones and xanthones depend on the number,

location, and nature of the substitutents. Therefore, calculations for such complicated compounds are difficult. Trends found for the influence of the substituents on the hydrophobicity of xanthones and flavones may improve the quality of the calculated hydrophobicity constants of new compounds.

EXPERIMENTAL

Absorption spectra were recorded on a Perkin–Elmer UV/Vis spectrometer in the range 230–500 nm and on an SF-26 spectrophotometer. PMR and ^{13}C NMR spectra of the synthesized compounds were recorded in $CDCl_3$ (for **1**, **3**, and **5**) and DMSO-d_6 (for **4**, **6**, **17**, **18**) on Bruker DPX-400 and Bruker AV-400 spectrometers (400.13 and 100.61 MHz, respectively). IR spectra were taken on a Bruker IFS 25 IR spectrometer in the range 700–4,000 cm^{-1}.

1,7-Dihydroxy-3,8-dimethoxyxanthone (gentiacaulein) (**7**) was isolated from *Gentianopsis barbata* [14] and graciously supplied by G. G. Nikolaeva. Its physicochemical properties agreed with those published [4].

1-Hydroxy-2,3,4,5-tetramethoxyxanthone (**8**) was isolated from *Halenia corniculata* and modified to 1-allyloxy-2,3,4,5-tetramethoxyxanthone (**9**) [2].

The other xanthones were synthesized by known methods.

Xanthone (**1**), mp 173–174°C, obtained by fusing *o*-hydroxybenzoic acid and phenol [15].

Condensation of *o*-hydroxybenzoic acid and resorcinol (under various conditions) gave 1-hydroxyxanthone (**3**), mp 147–148°C [16] and 3-hydroxyxanthone (**4**), mp 245–246°C [17].

1,3-Dihydroxyxanthone (**6**), mp 254–256°C, obtained from *o*-hydroxybenzoic acid and phloroglucinol [16].

4-Methoxyxanthone (**5**), mp 175–176°C, obtained by Ulmann condensation from guaiacol and 2-iodobenzoic acid [18] with subsequent cyclization of the diaryl ether in acetylchloride in the presence of H_2SO_4 [19].

The synthesized xanthones were purified by recrystallization and flash chromatography over a dry column containing TLC silica gel (Lachema L 5/40 μm). Compounds **3** and **6** were eluted by hexane:$CHCl_3$ with increasing concentration of the latter. Compound **4** was purified by elution with $CHCl_3$. The diaryl ether in the synthesis of **5** was isolated from the reaction mixture by elution with hexane:Et_2O with increasing fraction of the latter. The purity of the synthesized xanthones was determined using analytical (TLC using $CHCl_3$:EtOAc, 4:1; hexane:Et_2O, 7:3; and hexane:$CHCl_3$, 1:1) and spectral (IR, UV, PMR, and ^{13}C NMR spectroscopy) data. Physicochemical properties of synthesized xanthones **1** and **3**–**6** agreed with the literature values [9, 20–22].

The syntheses of 5,4′-dihydroxyflavone (**17**) and 3-hydroxy-7-methoxyflavone (**18**) have been published [6]. 5,7-Dihydroxyflavone (**16**) (Lancaster) was used without further purification.

Determination of Hydrophobicity Constants. The distribution of the studied compounds was studied using octanol–water by the previously developed method [8]. Stock solutions of compounds (with concentrations 10^{-2}–10^{-4} M) were prepared in octanol and shaken with a known volume of extractant (doubly distilled water saturated with octanol). The aqueous phase was acidified with HCl (1 N) to pH 2-3 in order to avoid dissociation for the study of the distribution of compounds containing free OH groups. Experiments were conducted with varying phase (octanol–water) ratios (1:20, 1:30, 1:40, 1:50, 1:60) at 20–22°C.

Optical density of the aqueous phase was measured on an SF-26 spectrophotometer in 2-, 5-, and 10-cm cuvettes; of the organic phase, in 0.005-, 0.05-, and 0.02-cm cuvettes.

The statistical significance of the resulting hydrophobicity constants was estimated using the mean-square deviation and the confidence interval calculated at the 95% probability limit using the literature formulas [23].

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