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Structure-Property Influence on the Amphiphilicity of Phenolipids †

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Abstract: In recent years, increasing interest has been observed in phenolipids used for enhancing the quality of products containing lipids in the food, pharmaceutical and cosmetic industries. A better understanding of the physicochemical properties of these amphiphilic compounds is crucial to maximizing their antioxidant and antiproliferation properties. Therefore, certain p-hydroxycinnamic acid derivatives were synthesised and their lipophilicity expressed as a partition coefficient (Log P) was measured using the shake-flask method. Additionally, the obtained results were compared with the calculated data in ALOGPS 2.1. An increase in lipophilicity was observed along with an increase alkyl chain length. Moreover, hydrophilic/hydrophobic properties are closely related with number substituents, especially hydroxyl group, in aromatic rings.

Keywords: phenolipids; ALOGPS 2.1; partition coefficient

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1. Introduction

Phenolipids are amphiphilic antioxidants with both hydrophilic phenolic moiety and hydrophobic molecules. These compounds should maintain the original functional properties of their parent compounds such as: antioxidant, chelating, free radical scavenging, antiallergic, anti-inflammatory, antimicrobial, antiviral and anticarcinogenic properties. These properties, especially antioxidant properties, are mainly associated with the number and distribution of hydroxyl groups in the aromatic rings of phenolic acid [1]. Alkyl esters of phenolic acids are the main known phenolipids. These compounds can be considered as potential replacements for synthetic antioxidants such as butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) commonly used in the fat and cosmetic industry [2]. Therefore, it is desirable gain a better understanding of the physicochemical properties of phenolic acid esters.

One of the most important parameters used to predict information regarding physicochemical properties is the partition coefficient (log P). This parameter allows to predict lipophilicty and quantitative structure-activity relationships (SAR) of bioactive compounds [3,4]. Therefore, log P is widely used in pharmaceutical industry to check the efficiency of the proposed drug such as: achievement of the target and binding at the target [5]. The most popular method for experimental determination of log P is the shake-flask method, in which the sample is partitioned between organic (n-octanol) and water (or aqueous buffer) phases in a flask. Next, the concentration of analyte in both the organic and water phases is quantified by: UV-VIS spectroscopy or a different method of spectrometric detection, high-performance liquid chromatography (HPLC) or gas chromatography (GC) [3]. However, to avoid time-consuming and labor-intensive experimental methods, computer programs were developed to estimate log P.

The aim of this study was to estimate the lipophilicity of certain phenolipids, p-hydroxycinnamic acid (HCA) derivatives, especially sinapic acid alkyl esters. Lipophilicity was expressed as a log P and measured using the shaking-flask method. Additionally, the obtained results were compared with calculated data in ALOGPS 2.1.

2. Materials and Methods

2.1. Reagents

All reagents, reactants, and solvents were purchased from Merck (Warsaw, Poland).

2.2. Synthesis of Phenolic Acid Esters

Synthesis procedures of esters: ethyl sinapate (ESA), octyl sinapate (OSA), cetyl sinapate (CSA), octyl caffeate (OCA) and octyl ferulate (OFA) were described in our previous studies [2].

2.3. Calculation of Partition Coefficients (log P) for Phenolic Compounds

2.3.1. Shake-Flask Method

Phenolic antioxidants: SA, ESA, OSA, CSA, CA, OCA, FA, OFA and BHA were diluted in the octanol and water phase. The prepared solutions were placed in an ultrasonic cleaner bath (Sono Swiss, SW 6H, Labo Plus, Warsaw, Poland) with ultrasound input power of 180 kW for 15 min due to enhanced solubility. The UV spectra of the analysed phenolic compounds were recorded using a Hitachi U-2900 spectrophotometer (Tokyo, Japan) in a 1 cm quartz cell in the octanol and water phases in order to find the characteristic band of the studied compounds. Next, calibration curves were constructed by plotting the concentrations as a function of UV absorbance values in the ranges for organic phase: 2.97×10^{-2}–2.23×10^{-1} μmol/mL, 4.71×10^{-2}–2.35×10^{-1} μmol/mL, 2.05×10^{-2}–1.74×10^{-1} μmol/mL, 2.00×10^{-2}–1.70×10^{-1} μmol/mL, 3.50×10^{-2}–2.97×10^{-1} μmol/mL, 2.55×10^{-2}–2.55×10^{-1} μmol/mL, 4.08×10^{-2}–2.04×10^{-1} μmol/mL, 4.72×10^{-2}–4.01×10^{-1} μmol/mL, 3.75×10^{-1}–1.88 μmol/mL and for water phase: 3.37×10^{-2}–2.36×10^{-1} μmol/mL, 3.59×10^{-2}–2.15×10^{-1} μmol/mL, 2.94×10^{-2}–1.96×10^{-1} μmol/mL, 2.12×10^{-2}–1.42×10^{-1} μmol/mL, 1.28×10^{-2}–1.03×10^{-1} μmol/mL, 1.30×10^{-1}–2.60×10^{-1} μmol/mL, 1.18×10^{-2}–4.71×10^{-2} μmol/mL, 1.46×10^{-1}–2.63×10^{-1} μmol/mL, 2.52×10^{-2}–2.52×10^{-1} μmol/mL SA, ESA, OSA, CSA, CA, OCA, FA, OFA and BHA, respectively.

1-Octanol–water distribution coefficients were determined using the shake flask method according to Guideline For The Testing Of Chemicals [6]. Briefly, selected phenolic compounds were weighed at a concentration within the calibration curve and dissolved in previously saturated two-phase solutions in a 50 mL conical flask. Then, the prepared solutions were shaken 250 cycles/min for 6 h using an orbital shaker (SHKA25081 CE, Labo Plus, Warsaw, Poland) and then the mixtures were left to stand for 6h to be partitioned between two phases. The absorbance of both phases was measured. The patriation coefficients were calculated according to Equation (1).

$$P_{o/w} = \frac{c_{n-octanol}}{c_n} \quad (1)$$

In which:

$C_{n\text{-}octanol}$—concentration of phenolic compounds in n-octanol,

C_n—concentration of phenolic compounds in water [6].

2.3.2. Theoretical Calculation

Log P values were also calculated using the ALOGPS 2.1 online program at the Virtual Computational Chemistry Laboratory [http://www.vcclab.org/lab/alogps/]. This program simulates participation in an n-octanol: water system.

2.4. Statistical Analysis

The log P values were determined three times within one day using the shake-flask method. The obtained results were presented as mean (c) ± standard deviation (SD).

3. Results

The lipophilicities of selected phenolic compounds are presented in Table 1.

Table 1. Log P_{exp} and calculated log P_{ALOGPs} values for HCA derivatives.

Phenolic Compound	Log P_{exp} ± SD	Log P_{ALOGPs}
SA	0.98 ± 0.05	1.26
ESA	3.87 ± 0.13	2.60
OSA	5.20 ± 0.24	5.34
CSA	7.63 ± 0.39	8.87
CA	0.99 ± 0.12	0.94
OCA	4.75 ± 0.05	5.02
FA	1.12 ± 0.07	1.25
OFA	5.72 ± 0.04	5.32
BHA	3.64 ± 0.05	3.15

Values are means (n = 3) ± standard deviations (SD).

It is noteworthy that, log P obtained for the HCA derivatives both by calculation (Log P_{ALOGPs}) and experimentally determined using the shake-flask method (log P_{exp}) are similar. As can be seen in Figure 1, the relationship between Log P_{exp} and Log P_{ALOGPs} can be expressed using the following linear regression Equation (2):

$$\text{Log } P_{ALOGPs} = 1.0773 \text{ Log } P_{exp} \quad (2)$$

The correlation coefficient r was 0.9678 and $r^2 = 0.9367$

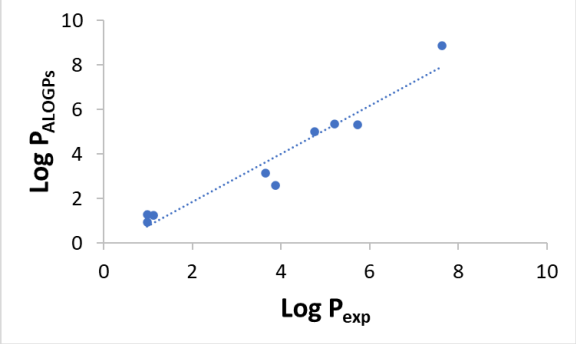

Figure 1. The relationship between log P_{ALOGPs} values and log P_{exp} values of the tested HCA derivatives.

In general, esterification of phenolic acids with alkyl alcohols increased the lipophilicity of the synthesised phenolipids (log P_{ALOGPs} = 0.94–1.26; log P_{exp} = 0.98–1.12 and log P_{ALOGPs} = 2.60–8.87; log P_{exp} = 3.87–7.63 for phenolic acids and their alkyl esters, respectively). As seen in Table 1, the lipophilicty of the HCA derivatives are well correlated with their structural features. The log P values increased along with the elongation of the alkyl ester side-chain. The same tendency was observed by Gaspar et al. [7] for SA derivatives and by Garrido et al. [8] for FA and CA derivatives with different acyl donor chain lengths from C1 to C4 and in our previous work. Additionally, the obtained log P values for tested HCA demonstrated that lipophilicity is closely related with the number of hydroxyl group substitutions in aromatic rings. The Log P_{ALOGPs} values for CA was the lowest (Log P_{ALOGPs} = 0.94) in comparison with FA (log P_{ALOGPs} = 1.25) and SA (log P_{ALOGPs} = 1.26). The same

tendency was observed in the tested phenolic acid octyl ester—log P_{ALOGPs} were 5.02, 5.32 and 5.34 for OCA, OFA and OSA, respectively. Surprisingly, there were no differences between log P_{exp} for CA and SA (Δlog P_{exp} = 0.01). Moreover, the obtained data demonstrated for both series (phenolic acids and octyl esters) slight differences in lipophilicity (log P_{ALOGPs} differences between SA and FA 0.01 and log P_{ALOGPs} differences between OSA and OFA 0.02).

Food additives BHA had log P_{exp} = 3.64 and Log P_{ALOGPs} = 3.15. Therefore, there is more hydrophobic than phenolic acid but less than in the obtained HCA derivatives, except for ESA.

4. Conclusions

The obtained data in this study proves that the computational method of log P is convenient for estimating the lipophilicity of HCA derivatives. The esterification of phenolic acid with alcohol effectively increased their lipophilicity. Furthermore, log P values of phenolipids depend on the length of the alkyl-ester side chain and the number of hydroxyl groups in aromatic rings.

Log P allows to predict overall physicochemical parameters of new functionalized compounds such as phenolipids due to its correlation with antioxidant and cytotoxic activates [4,7]. The higher lipophilicity of antioxidants is often desired in fat-based products, because it changes the absorption and distribution properties of HCA derivatives. Increasing lipophilicity increased the ability of phenolipids to achieve local concentration at water-lipid interface, where lipid oxidation started, through free radical attack from the aqueous phase [4,7]. Therefore, phenolipids can be used as an effective additive to food-based products to prevent oxidation.

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