Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 686–691

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Lipophilicity of some GABAergic phenols and related compounds determined by HPLC and partition coefficients in different systems

Gabriela N. Reiner, Diana O. Labuckas, Daniel A. García*

Departmento de Química - Instituto de Ciencia y Tecnología de los Alimentos (ICTA), Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sarsfield 1611, 5016 Córdoba, Argentina

ARTICLE INFO

Article history:
Received 18 November 2008
Received in revised form
22 December 2008
Accepted 23 December 2008
Available online 8 January 2009

Keywords:
GABAergic phenols
Lipophilicity
IAM–HPLC
Partition coefficient
Liposomes
Correlation analysis

ABSTRACT

Some phenolic compounds, like propofol and thymol, have been shown to act on the $GABA_A$ receptor. Taking into account the hydrophobicity of these compounds, their interaction with the membrane surrounding the receptor and consequent non-specific effect on receptor modulation cannot be neglected. In the present work, we determined and correlated several lipophilic parameters for both GABAergic agents and three other related phenolic compounds (eugenol, carvacrol and chlorothymol), including $\log P_{o/w}$, retention data in high performance liquid chromatography (HPLC) by using C18 and immobilized artificial membrane (IAM) columns at different temperatures, and partition coefficients determined in phospholipid liposomes. The correlation results demonstrated the high capacity of the compounds assayed to interact with phospholipid membrane phases, which can be predicted by simple model systems as $\log P_{o/w}$ or HPLC. The values obtained by HPLC using a fast screening IAM column were the quantitatively closest to the partition coefficients determined in liposome systems, due to the capacity of this column to permit the establishment of molecular interactions like those found in phospholipid membranes. Finally, the fact that all the compounds studied are able to interact with membranes would suggest the participation of some alteration of the $GABA_A$ receptor lipid environment as part of the receptor modulation exerted by phenolic compounds.

1. Introduction

$GABA_A$ receptors are ligand-gated ion channels that mediate rapid synaptic inhibition in the central nervous system. They constitute the main inhibitory receptors of the Central Nervous System. The $GABA_A$ receptor possesses binding sites for drugs other than the neurotransmitter GABA, including benzodiazepines, barbiturates, and the convulsant picrotoxinin, which behave as allosteric modulators or channel blockers. A wide spectrum of drugs, toxic agents and metals modify $GABA_A$ receptor function by directly interacting either with these binding sites or with other as yet not well-described sites present in the receptor complex [1,2]. Propofol and thymol have been shown to act on this receptor as allosteric modulators at low concentrations or to have a direct effect on the channel opening at higher concentrations. These activities, or at least the former, are mediated by their interaction with a specific site in the $GABA_A$ receptor [3,4]. However, taking into account the lipophilicity of both compounds, their interaction with the membrane surrounding the receptor and a consequent non-specific effect on the receptor modulation cannot be discarded. It is known that the activity of this receptor may be affected by surface-active compounds and by physical changes in the membrane [5–9].

The molecular parameter "lipophilicity" is commonly associated with the *n*-octanol/water partition coefficient ($\log P_{o/w}$), which is widely accepted as an artificial measure of membrane partitioning since the pioneering studies of Hansch, Fujita and Leo [10,11]. Retention data from high performance liquid chromatography (HPLC) are other parameters often used to determine lipophilicity. The most common system is reverse phase HPLC (RP-HPLC), where hydrophobicity is the main force governing retention [12]. Recently, in the last two decades, biomimetic chromatographic partition systems have been utilized through immobilized artificial membranes (IAMs) as packing material in HPLC columns. IAM columns are prepared by covalent binding of a monolayer of phospholipids to silica particles mimicking the fluid lipid bilayer of biological membranes [13,14]. This method correlates well with partition in liposome systems [15–17]. Liposomes are very reliable models of biological membranes because they share several structural similarities [18]. However, the use of liposomes for predicting drug–membrane interactions is time-consuming and tedious, particularly for wide-scale studies.

Thus, in the present work we determined and correlated several lipophilic parameters for the GABAergic agents propofol and thymol, and three other related phenolic compounds

* Corresponding author. Tel.: +54 351 4344983; fax: +54 351 4334139.
E-mail address: dagarcia@efn.uncor.edu (D.A. García).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.12.040

(eugenol, carvacrol and chlorothymol, the activities of which on the $GABA_A$ receptor are being studied in our lab) (Fig. 1), including $\log P_{o/w}$, retention data in HPLC by using RP-C18 and IAM columns at different temperatures, and partition coefficients determined in liposome systems composed of soybean lecithin or dipalmitoyl phosphatidylcholine (dpPC). Taking into account that the IAM–HPLC column used in this study was a fast screening column, one of the aims was to analyze its predictive ability to estimate the interaction between the phenolic compounds with the partition coefficient corresponding to liposome systems.

2. Experimental

2.1. Materials

Propofol (2,6-bis(isopropyl)-phenol), thymol (5-methyl-2-isopropyl-phenol), carvacrol (2-methyl-5-isopropyl-phenol), eugenol (2-methoxy-4-prop-2-enyl-phenol), chlorothymol (5-methyl-4-chloro-2-isopropyl-phenol) and dpPC were obtained from Sigma Chemical Co. (St Louis, MO, USA). Soybean lecithin was the commercially available grade. All the other chemicals and solvents were of analytical or HPLC grade.

2.2. Spectrophotometric determinations

Aqueous solutions (with DMSO 0.25% v/v and adjusted at pH 7 with NaOH) of each phenolic compound at different final concentrations (0.0625–3 mM) were prepared and their absorbance spectra between 250 and 300 nm were determined with a Beckman DU 7500 (Fullerton, CA, USA) double beam spectrophotometer using a quartz cell. Calibration curves were obtained from the absorbance values at their respective λ_{max} as a function of different concentrations of each compound, and molar absorption coefficients (ε) were determined by adjusting a linear regression to the experimental data. The range of concentrations used for each compound depended on their solubility limits, which were calculated by the dilution of different quantities in the aqueous solutions used, incubation at room temperature for 30 min until equilibrium was reached, centrifugation at 30,000 × *g* for 15 min, and measuring the absorbance of supernatant at λ_{max}. The solubility limit was taken at the concentration point where linearity is lost.

2.3. Calculation of equilibrium dissociation constant

Aqueous solutions of the phenolic compounds (100 μM final concentration) were prepared at different pH values (between 6.5 and 12.5) measured with a Cole Parmer 59003 pH meter equipped with a glass electrode and a sensor for automatic temperature compensation. The pH was adjusted by adding HCl or NaOH. Absorption spectra were obtained against a blank of the same pH used for dissolving the compounds. The method used for calculation of the equilibrium dissociation constant was previously described [19,20]. Duplicate independent experiments were done for each compound.

2.4. HPLC

All retention measurements by HPLC were made with a PerkinElmer Series 200 HPLC apparatus (Shelton, CT, USA) equipped with a column oven for temperature control and UV detector set at 280 nm. An injector with 20 μl sample loop was used for all sample injections. Duplicate injections were performed.

2.4.1. IAM–HPLC method

Methanol solutions of 10 mM of each compound were diluted 1/10 with the solvent used as mobile phase and injected on a IAM-DD2 Fast Screen Mini Column (30 mm length and 4.6 mm i.d., Regis Technology, Morton Grove, IL, USA). The capacity factors (k) were calculated from the retention times as follows: $k = (t_r - t_0)/t_0$, where t_r is the retention time of the compound and t_0 is the dead time of the column, which is equal to the retention time of a substance which is not retained as citric acid used in the present work [15]. The experiments were performed with two different mobile phases: pure water or acetonitrile (ACN) 10% (v/v), and the flow rate was 0.2 ml/min. All compounds were assayed at 30 and 45 °C. The chromatographic system was allowed to equilibrate at each temperature for at least 15 min prior to every experiment.

2.4.2. RP-HPLC method

In *RP-HPLC* experiments the samples were diluted as explained for IAM–HPLC. The apparatus was equipped with a C18 reversed-phase HPLC column (120 mm × 4 mm i.d., Nucleosil ET250/8/4, Macherey-Nagel, Düren, Germany). Capacity factors (k) were calculated as detailed before and the retention data were determined at 30 and 45 °C. The mobile phase was ACN 50% (v/v) in bi-distilled water and the flow rate was 0.5 ml/min.

2.5. Measurement of $\log P_{o/w}$

Partition coefficients of the phenolic compounds in *n*-octanol/water system ($\log P_{o/w}$) were determined by a conventional shake-flask method. Briefly, each compound was dissolved in *n*-octanol (10 mM) and mixed with nine volumes of bi-distilled water. The mixture was vigorously shaken and after 15 min of incubation was centrifuged for 10 min at 350 × *g*. The absorbances at λ_{max} of the upper and the lower phases were measured against upper and lower phases of a similar *n*-octanol–water mixture without any compound. In order to determine the concentration in the aqueous phase of the most lipophilic compounds assayed, propofol and chlorothymol, their lower phases were separated, extracted with hexane, evaporated at 45 °C in a Büchi rotating evaporator and resuspended in a lesser volume of *n*-octanol for its spectrophotometric determination. All values correspond to the mean of 2–3 measurements.

$\log P_{o/w}$ values were also calculated by using the Osiris Property Explorer software (freely available in www.chemexper.com; ChemExper sprl, Court-st-Etienne, Belgium). The $\log P_{o/w}$ calculation method is implemented as an increment system adding contributions of every atom based on its atom type. All together,

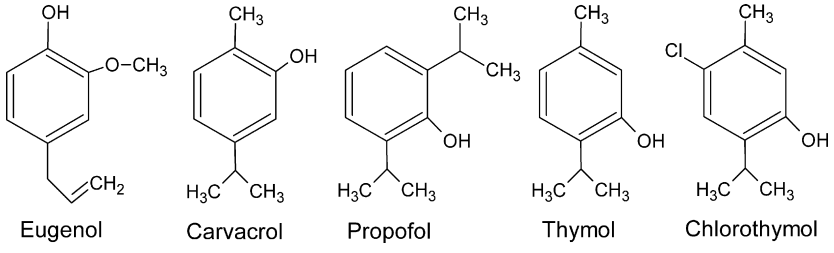

Fig. 1. Chemical structures of the phenolic compounds analyzed.

Table 1
Data obtained from spectrophotometric experiments.

Compound	λ_{max}	ε	Sol (mM)	p*Ka*
Eugenol	280	2667	16.0	10.72
Carvacrol	274	1204	10.0	11.02
Propofol	270	1100	2.5	11.59
Thymol	275	1212	10.0	11.11
Chlorothymol	282	1404	3.0	10.71

λ_{max}, molar absorption coefficients (ε: M^{-1} cm^{-1}) and aqueous solubility (Sol) were determined from experiments like shown in Fig. 2. p*Ka* values correspond to the theoretical fitting to the experimental data of absorbance vs. pH curves as was explained in Section 2 (see Fig. 3).

the log $P_{o/w}$ predicting engine distinguishes 368 atom types which are composed of various properties of the atom itself (atomic number and ring membership) as well as of its direct neighbours (bond type, aromaticity state and encoded atomic number).

2.6. Liposome-buffer partition coefficients

Liposomes were prepared by evaporating, under reduced pressure, a chloroform solution of lipids (lecithin or dpPC) as described before [21]. The dry lipid was suspended in buffer Tris–HCl 50 mM pH 7.4 by repeating six consecutive cycles of heating at 50 °C for 2 min and vortexing for 30 s. Before use, the suspension was diluted to a final lipid concentration of 3.75 mg/ml, and each phenolic compound was added at 0.5–1 mM (final concentration). The mixture was shaken vigorously for 5 min at room temperature and centrifuged at 30,000 × *g* for 15 min. The supernatant was carefully separated from the pellets, and the amount of the phenols in the solution was calculated from their absorbance values at λ_{max}. All values correspond to the mean of 2–6 independent experiments.

2.7. Correlation analysis

Principal component analysis (PCA) was used to obtain the correlation matrix of all pairs of variables and to know if the variability among the compounds is explained by only one or by more factors (principal components).

PCA was done as reported previously [22,23], using StatistiXL software (www.statistixl.com, Nedlands, Australia). Particularly, our data required standardization since the different properties were measured in non-comparable units. In order to do that, the original data (x_{ij}) were centred (transformed to deviation from the respective mean of the values of each *i* property for the *j*s compound (x_i)) and then standardized by dividing the centred data by the standard deviation of the values of the corresponding *i* property for the *j*s compound (σ_i) by applying the following calculation to each element of the original data matrix: $(x_{ij} - x_i)/\sigma_i$.

3. Results and discussion

Fig. 1 shows the chemical structure of phenolic compounds studied. Their absorption spectra at pH 7 and typical calibration and solubility curves are shown in Fig. 2. The corresponding λ_{max}, ε and aqueous solubility extracted from these types of curves are included in Table 1. The spectrophotometric data were used to quantify the compounds in the partition coefficient experiment as was explained in Section 2.

It is interesting to note that, in spite of the solubility data not being included in the correlation analysis, these showed a high negative correlation with all lipophilic parameters determined in the present study ($r^2 > -0.90$). This behaviour is understandable since aqueous solubility is an inverse measurement of compound hydrophobicity.

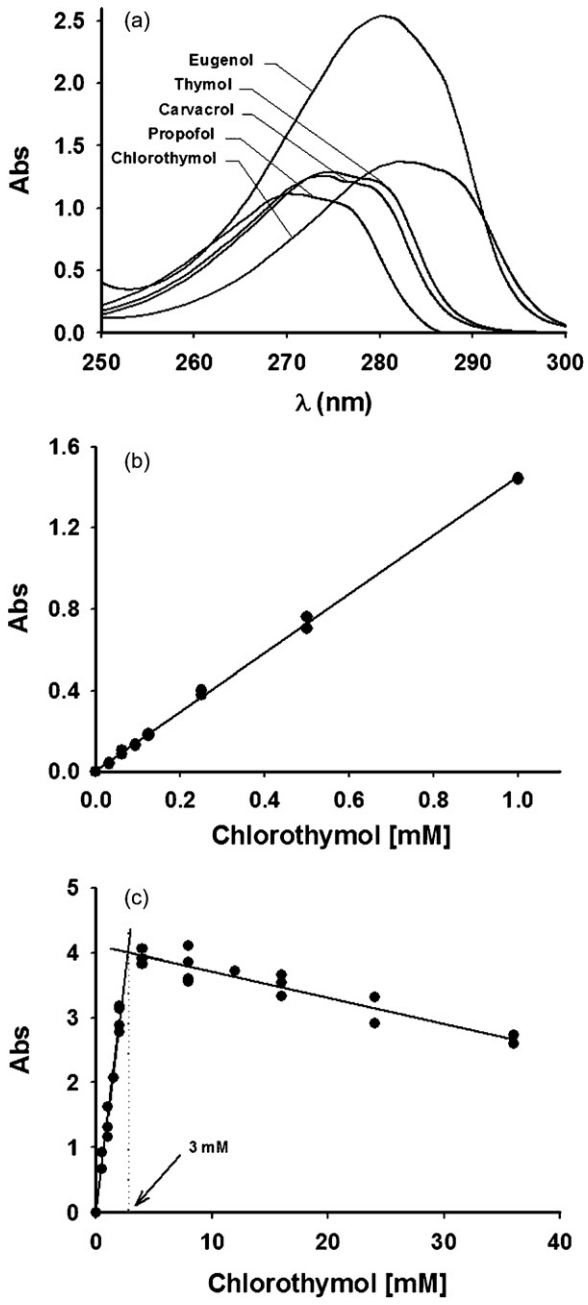

Fig. 2. UV absorbance spectra (a), typical calibration (b), and solubility (c) curves. Spectra were measured from aqueous solutions of phenolic compounds (1 mM, pH 7). Typical calibration curve represents the fitting of a linear regression to absorbance values measured at different concentrations of chlorothymol. The arrow in panel (c) indicates the aqueous solubility of chlorothymol determined as described in Section 2.

Fig. 3 shows a typical titration curve of a phenolic compound (chlorothymol) in water; points correspond to the experimental values of observed absorbance (A_{obs}) vs. pH as explained in Section 2. The theoretical fitting to the experimental data (A_{obs}) was good and allowed the calculation of p*Ka* values ranging between 10.7 and 11.6 according to each compound (Table 1).

In the present work, we evaluate the use of a HPLC–IAM Fast Screen Mini column to predict the membrane interaction of some lipophilic compounds, in particular phenolic GABAergic and related compounds, in order to gain a deeper insight into their non-specific effects involved in the modulation of a membrane integral protein

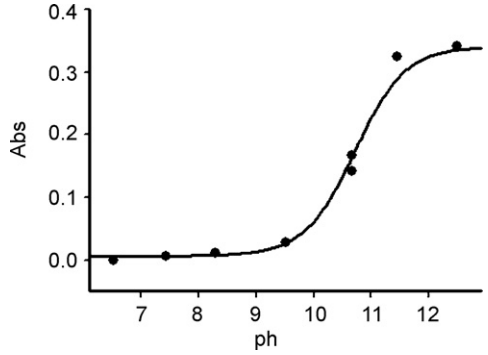

Fig. 3. Typical titration curve of absorbance as a function of pH. UV absorbance of chlorothymol (100 μM) at 302 nm corresponding to the λ_{max} of the dissociated specie (pH 12.5). Line corresponds to the curve calculated according to the theoretical considerations previously described [19,20].

such as the $GABA_A$ receptor. The main advantages of this column are its low cost, simplicity and very short analysis time [15,24].

All data related to the lipophilicity of the compounds obtained in the present work are shown as logarithmic values in a data matrix in Table 2. All these data were submitted to the correlation analysis as explained in Section 2.

Log *k* values obtained by the IAM–HPLC method ($\log k_{IAM}$) for the five compounds, not only by using two different mobile phases but also at two different temperatures, showed the following order of increasing affinity for immobilized phospholipids: eugenol < thymol ≈ carvacrol < propofol < chlorothymol (Table 2). Log k_{IAM} values corresponding to ACN 10%, and consequently the corresponding retention times, were lower than those obtained in pure water, as expected considering the elevated hydrophobicity of the assayed compounds [14,25]. Mixtures of water with methanol or ACN at different proportions are the most commonly used mobile phases in liquid chromatography [18,26]. In this work, we chose ACN–water mixture, taking into account that no significant difference had been previously observed between *k* values extrapolated from either methanol or ACN and that methanol as a cosolvent causes more lipid leaching in IAM columns [18,27]. Fig. 4 shows that k_{IAM} values observed at 45 °C were higher than those at 30 °C. This behaviour suggests that the solute–immobilized phospholipid interaction is favoured by a temperature increase. Previous reports of our group support the present results, demonstrating that the partition of lipophilic drugs in the membrane increases in quantity and depth as the structural order of the membrane diminishes by effect of the temperature increase [20,28]. Thus, it is probable that, regardless of the phospholipids in IAM columns being immobilized by means of the binding of their hydrocarbon chains to the silica particles, the temperature increase would induce a more disordered interface and consequently a more extensive interaction with lipophilic solutes. All $\log k_{IAM}$ values of the assayed compounds presented a very high correlation with all other lipophilic parameters, including RP-HPLC data and partition coefficients in different systems, as shown in the correlation matrix (Table 3).

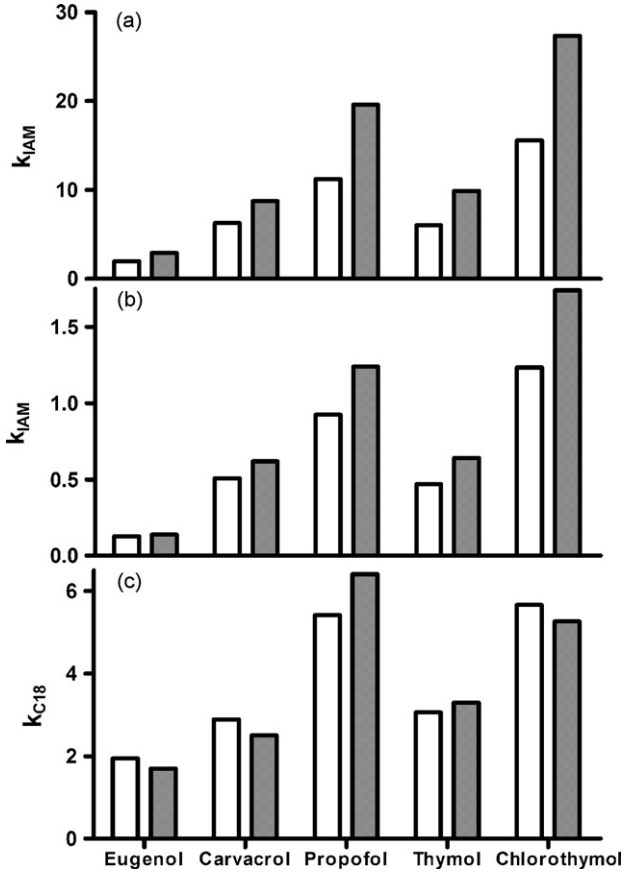

Fig. 4. HPLC capacity factor (*k*) of phenolic compounds at different temperatures. The *k* values were determined: (a) with IAM column (k_{IAM}) using water as mobile phase, (b) with IAM column (k_{IAM}) using ACN 10% as mobile phase and (c) with C18 reverse phase column (k_{c18}) using ACN 50% as mobile phase. The running temperatures were adjusted to 30 °C (white bars) or 45 °C (grey bars).

Table 2
Original data matrix.

	Eugenol	Carvacrol	Propofol	Thymol	Chlorothymol
$\log k_{IAM-W}$ (30 °C)	0.297	0.797	1.052	0.780	1.193
$\log k_{IAM-W}$ (45 °C)	0.463	0.942	1.293	0.995	1.437
$\log k_{IAM-A10}$ (30 °C)	−0.896	−0.292	−0.035	−0.327	0.092
$\log k_{IAM-A10}$ (45 °C)	−0.856	−0.207	0.093	−0.191	0.240
$\log k_{C18}$ (30 °C)	0.291	0.461	0.733	0.486	0.735
$\log k_{C18}$ (45 °C)	0.232	0.399	0.807	0.518	0.722
$\log P_{o/w}$ exp	2.297	3.140	3.810	3.210	3.820
$\log P_{o/w}$ calc	2.420	3.090	3.870	3.090	3.710
$\log K_W$ (30 °C)	1.791	2.291	2.546	2.274	2.687
$\log P_{lec-b}$	2.203	2.760	2.895	2.902	3.171
$\log P_{dpPC-b}$	1.890	2.480	3.062	2.637	3.539

All data were determined or calculated in the present work according to Section 2. For IAM–HPLC variables, the mobile phase used is indicated: water (W) or ACN 10% (A10). Log *P* indicate partition coefficients in different systems: *n*-octanol/water (o/w), lecithin liposomes/buffer (lec-b) and dpPC liposomes/buffer (dpPC-b). Exp and calc correspond to experimental and calculated data, respectively.

The *k* value is linearly related to the equilibrium partition coefficient (*K*) of a solute that partitions between the stationary phase and the mobile phase according to: $k = (V_m/V_s)K$, where V_m is the volume of the mobile phase corresponding to the total volume of solvent within the HPLC column and calculated as: $V_m = f_r t_0$, f_r being the flow rate. V_s, the stationary phase, is the volume of the interface created by the immobilized phospholipids and, in this work, it was calculated according to the data given by Taillardat-Bertschinger et al. [17]. IAM columns and fluid phospholipid membranes are physically similar. IAM columns exhibit interface motion properties similar to those of mobile lipids in liposomes, as was described by ^{31}P NMR studies [29]. Thus, it would be expected that *K* from immobilized phosphatidylcholine (IAM columns) would represent the closest values to the partition coefficients in dpPC liposomes. In this aspect, the values of log *K* obtained were slightly lower than the log of partition coefficients in liposomes, but this difference was more noticeable for the most lipophilic compounds (Table 2). Perhaps this variation is because liposomes are formed by a bilayer of phospholipids that separates the external and the internal aqueous phase, while IAM surfaces are a solid phase with only a monolayer of phospholipids bonded to silica particles. Furthermore, the den-

Table 3
Simple correlation matrix.

Variables	$\log k_{IAM-W}$ (30 °C)	$\log k_{IAM-W}$ (45 °C)	$\log k_{IAM-A10}$ (30 °C)	$\log k_{IAM-A10}$ (45 °C)	$\log k_{C18}$ (30 °C)	$\log k_{C18}$ (45 °C)	$\log P_{o/w}$ exp	$\log P_{o/w}$ calc	$\log K_W$ (30 °C)	$\log P_{lec-b}$	$\log P_{dpPC-b}$
$\log k_{IAM-W}$ (30 °C)	1.000	–	–	–	–	–	–	–	–	–	–
$\log k_{IAM-W}$ (45 °C)	0.997	1.000	–	–	–	–	–	–	–	–	–
$\log k_{IAM-A10}$ (30 °C)	0.998	0.992	1.000	–	–	–	–	–	–	–	–
$\log k_{IAM-A10}$ (45 °C)	0.997	0.993	0.999	1.000	–	–	–	–	–	–	–
$\log k_{C18}$ (30 °C)	0.963	0.976	0.951	0.950	1.000	–	–	–	–	–	–
$\log k_{C18}$ (45 °C)	0.920	0.943	0.912	0.916	0.977	1.000	–	–	–	–	–
$\log P_{o/w}$ exp	0.988	0.991	0.988	0.989	0.975	0.962	1.000	–	–	–	–
$\log P_{o/w}$ calc	0.961	0.967	0.958	0.955	0.985	0.980	0.987	1.000	–	–	–
$\log K_W$ (30 °C)	1.000	0.997	0.998	0.997	0.963	0.920	0.988	0.961	1.000	–	–
$\log P_{lec-b}$	0.957	0.953	0.960	0.969	0.868	0.833	0.931	0.862	0.957	1.000	–
$\log P_{dpPC-b}$	0.974	0.984	0.959	0.963	0.967	0.914	0.954	0.927	0.974	0.936	1.000

The values represent the result of a simple correlation among each pair of variables and indicate the correlation coefficients (r). It is only considered the matrix inferior triangle (under the diagonal). The elements of the diagonal equal 1 because each variable correlates perfectly with itself. The upper triangle is a reflected image of the inferior triangle, because r is a symmetric association measure. See variable names in Table 2.

sity of phospholipid molecules is higher in liposomes than in IAM columns. The surface area per lipid headgroup is around 62 Å^2 in liposomes and 105 Å^2 in double-chain IAM columns (see Taillardat-Bertschinger et al. [18] and refs. therein). Nevertheless, the log K values showed a very good linear correlation with the logs of partition coefficients determined in dpPC liposome systems ($\log P_{dpPC-b}$) ($r^2 = 0.95$) (Table 3 and Fig. 5). Values of log partition coefficients of propofol obtained using lecithin or dpPC liposomes ($\log P_{lec-b}$: 2.89 and $\log P_{dpPC-b}$: 3.06) (Table 2) were comparable to those described before using egg-PC or dimyristoyl-PC liposomes (2.60 and 2.90, respectively) [30].

The $\log P_{o/w}$ has been used as a measure of hydrophobicity of different compounds constituting an important tool in structure–activity relationship studies [31,32]. Its direct determination, however, is not usually applicable to highly lipophilic compounds ($P_{o/w} > 3$) because of their extremely low concentrations in water. For this reason, in this work we did a re-extraction of the most lipophilic compounds (propofol and chlorothymol) from the aqueous phase to get a higher concentration sample. The $P_{o/w}$ experimental values determined in the present study were similar to the values calculated by using specific software (see Section 2) and other empirical data described before [32,33].

Log k values of the phenolic compounds determined by RP-C18 column ($\log k_{C18}$) showed a comparable relative lipophilicity sequence to that described before by using the IAM column, with the exception that propofol and chlorothymol presented very similar $\log k_{C18}$ values. This lipophilicity arrangement was maintained at both assay temperatures and no clear general effect of the temperature on $\log k_{C18}$ was found (temperature increase produced small decrements of $\log k_{C18}$ of eugenol, carvacrol and chlorothymol, but increments of the values corresponding to propofol and thymol) (see the corresponding k values in Fig. 4). Nevertheless, $\log k_{C18}$ values correlated very well with all $\log k_{IAM}$ values and with the partition coefficients in all systems including $\log P_{o/w}$. Some previous studies have revealed that the relationship between $\log P_{o/w}$ and $\log k_{C18}$ usually requires some correction terms for hydrogen-bonding effects [34]. It was observed that the hydrogen bond acidity strongly affects the reversed phase retention, but it does not have a significant effect on the n-octanol/water partition [12]. However, considering that the pKa values of the five phenolic compounds obtained in this work are very similar (values range between 10.7 and 11.6) (Table 1) and far from the pH of solutions used, differences between their hydrogen bonding effects would be negligible. It should also be considered that the pKa of the compound to analyze is affected by the permittivity of the mobile phase. This means that this parameter changes according to the mixture of organic solvent with water. Dissociation of substances in ACN–water mixtures is regulated by electrostatic interactions and specific solvent–solute interactions. In the dissociation of neutral or anion acids, charges are created and the dissociation process is disturbed when the relative permittivity of the medium decreases with the increase in ACN content (relative permittivity of ACN and water: 38.8 at 20 °C and 78.5 at 25 °C, respectively). Hence, for the dissociation of phenolic protons, the electrostatic interaction exceeds the specific solvation and the pKa increases with the percentage of ACN in the mixture (see Pissinis et al. [35] and references therein). According to this, it would be expected that the presence of ACN in our experiments would increase the phenolic pKa values even more, highlighting the fact that, under the present experimental conditions, there is neither ionization of any compounds nor charge effects.

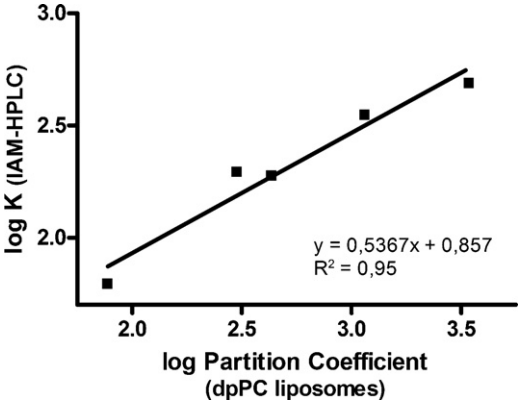

Fig. 5. Correlation of logarithmic values of the equilibrium partition coefficient (K) with the partition coefficients in dpPC liposomes. Log K were determined by HPLC using IAM column at 30 °C with water as mobile phase, as explained in the text. All data corresponding to the five phenolic compounds were taken from Table 2.

Taking into account the values of all lipophilic parameters obtained, eugenol showed the lowest lipophilicity while thymol and carvacrol displayed intermediate values. Comparing the two most hydrophobic compounds assayed, chlorothymol and propofol, the former showed clearly higher values of $\log k_{IAM}$ and partition coefficients in liposomes, while both presented very similar values of $\log k_{C18}$ and $\log P_{o/w}$. The difference between the relative values obtained by using two types of HPLC columns may be explained by the fact that the C18-RP column, for example, retains analytes solely on the basis of hydrophobicity, while IAM more closely mimics the interaction of analytes with biological membranes, where a combination of hydrophobic, ion-pairing, and hydrogen-bonding interactions are possible. This combination of interactions measured by the IAM column is known as phospholipophilicity [36]. The higher partition coefficient obtained for chlorothymol respect to

Table 4
Explained variance.

Value	PC1	PC2	PC3
Eigenvalue	10.61	0.27	0.07
% of variability	96.46	2.47	0.68
Cumulative %	96.46	98.93	99.61

The eigenvalues for each principal component (PC), the percentages of total variability explained for each PC and the cumulative percentage are shown. Calculations were performed as described in Section 2.

propofol, in phospholipids involving systems which contain phosphatidylcholine, could be due to the relatively greater ability of the former to establish attractive electrostatic interactions with the positive electric charge of the N atom of the phosphatidylcholine polar head. This charge–dipole intermolecular interaction is expected to be enhanced as the molecules present larger dipole moment. In this sense, although both compounds present permanent dipoles, chlorothymol possesses a dipole moment magnitude superior to that of propofol (2.096 vs. 1.357 debyes, respectively; values calculated with the ChemDraw program, CambridgeSoft Corporation, Cambridge, UK). The less marked difference between the $\log P_{\text{lec-b}}$ values of propofol and chlorothymol respect to their $\log P_{\text{dpPC-b}}$ values, may be attributable to the fact that lecithin composition is a mixture of fatty acids, glycerol, glycolipids and triglycerides with a relative high proportion of phospholipids (∼40%*), including mainly phosphatidylcholine (∼15%*), phosphatidylethanolamine, phosphatidylinositol and phosphatidic acid (*data given by the commercial company).

Finally, considering that the first principal component explains more than 96% of the total variability (Table 4) and that all measured variables contribute almost exclusively to this first principal component (according to the eigenvector values, results not shown), it is demonstrated that only one factor is responsible for the total variability among the assayed compounds. This factor is the hydrophobicity or the lipophilicity of each compound, which was quantified by the value of the variables measured or calculated in the present study.

4. Conclusion

The correlation results demonstrate that the capacity of the compounds assayed to interact with phospholipid membrane phases can be predicted by other simpler model systems such as $\log P_{\text{o/w}}$, RP-HPLC and IAM–HPLC. Since the high correlations obtained were reached mainly because of the non-charged state of all the assayed compounds under the experimental conditions, we think that these circumstances should be taken into account for correct future predictions. In this sense, it will be interesting to increase the number of compound to be evaluated in order to generalize these findings to other related molecules. The values obtained by HPLC using a fast screening IAM column were those quantitatively closest to the partition coefficients determined in liposome systems, due to its capacity to permit establish molecular interactions like those existent in phospholipid membranes. Finally, the present results demonstrate that all the compounds studied are able to interact with membranes, which would suggest the participation of some alteration of the $GABA_A$ receptor lipid environment as part of the receptor modulation exerted by phenolic compounds.

Acknowledgments

This work was partially supported by grants from SECyT-Universidad Nacional de Córdoba, CONICET (Argentina), FONCyT (Argentina) and CAEN-ISN. D.A.G. is a member of CONICET (Argentina) and G.N.R. holds a fellowship from the same institution.

References

[1] R.L. MacDonald, R.W. Olsen, Annu. Rev. Neurosci. 17 (1994) 569–602.
[2] U. Rudolph, H. Möhler, Annu. Rev. Pharmacol. Toxicol. 44 (2004) 475–498.
[3] B. Mohammadi, G. Haeseler, M. Leuwer, R. Dengler, K. Krampfl, J. Bufler, Eur. J. Pharmacol. 421 (2001) 85–91.
[4] D.A. García, J. Bujons, C. Vale, C. Suñol, Neuropharmacology 50 (2006) 25–35.
[5] M.R. Witt, M. Nielsen, J. Neurochem. 62 (1994) 1432–1439.
[6] D.A. García, M.A. Perillo, J.A. Zygadlo, I.D. Martijena, Lipids 30 (1995) 1105–1110.
[7] M.A. Perillo, D.A. García, R.H. Marín, J.A. Zygadlo, Mol. Membr. Biol. 16 (1999) 189–194.
[8] M. Pytel, K. Mercik, J.W. Mozrzymas, Br. J. Pharmcol. 148 (2006) 413–422.
[9] R. Søgaard, T.M. Werge, C. Bertelsen, C. Lundbye, K.L. Madsen, C.H. Nielsen, J.A. Lundbæk, Biochemistry 45 (2006) 13118–13129.
[10] C. Hansch, P.P. Mahoney, T. Fujita, R.M. Muir, Nature 194 (1962) 178–180.
[11] A. Leo, C. Hansch, D. Elkins, Chem. Rev. 71 (1971) 525–616.
[12] K. Valko, C.M. Du, C. Bevan, D.P. Reynolds, M.H. Abraham, Curr. Med. Chem. 8 (2001) 1137–1146.
[13] C. Pidgeon, U.V. Venkataram, Anal. Biochem. 176 (1989) 36–47.
[14] F. Darrouzain, P. Dallet, J.P. Dubost, L. Ismaili, F. Pehourcq, B. Bannwarth, M. Matoga, Y.C. Guillaume, J. Pharm. Biomed. Anal. 41 (2006) 228–232.
[15] C. Ottiger, H. Wunderli-Allenspach, Pharm. Res. 16 (1999) 643–650.
[16] T. Osterberg, M. Svensson, P. Lundahl, Eur. J. Pharm. Sci. 12 (2001) 427–439.
[17] A. Taillardat-Bertschinger, C.A. Martinet, P.A. Carrupt, M. Reist, G. Caron, R. Fruttero, B. Testa, Pharm. Res. 19 (2002) 729–737.
[18] A. Taillardat-Bertschinger, P.A. Carrupt, F. Barbato, B. Testa, J. Med. Chem. 46 (2003) 655–665.
[19] D.A. García, M.A. Perillo, Biochim. Biophys. Acta 1324 (1997) 76–84.
[20] D.A. García, M.A. Perillo, Biochim. Biophys. Acta 1418 (1999) 221–231.
[21] M.A. Perillo, D.A. García, Colloid. Surf. B: Biointerfaces 20 (2001) 63–72.
[22] F.J. Rohlf, Numerical Taxonomy and Multivariate Analysis System, Exeter Publishing, New York, 1984.
[23] M.A. Perillo, D.A. García, A. Arce, Mol. Membr. Biol. 12 (1995) 217–224.
[24] S. Ong, C. Pidgeon, Anal. Chem. 67 (1995) 2119–2128.
[25] A.B. Hameda, S. Elosta, J. Havel, J. Chromatogr. A 1084 (2005) 7–12.
[26] A. Alvarez-Zepeda, B.N. Barman, D.E. Martire, Anal. Chem. 64 (1992) 1978–1984.
[27] J. Li, J. Sun, Z. He, J. Chromatogr. A 1140 (2007) 174–179.
[28] D.A. García, M.A. Perillo, Colloid. Surf. B: Biointerfaces 9 (1997) 49–57.
[29] S. Ong, X. Qiu, C. Pidgeon, J. Phys. Chem. 98 (1994) 10189–10199.
[30] F. Momo, S. Fabris, A. Bindoli, G. Scutari, R. Stevanato, Biophys. Chem. 95 (2002) 145–155.
[31] C. Hansch, T. Fujita, J. Am. Chem. Soc. 86 (1964) 1616–1626.
[32] C. Hansch, A. Leo, Exploring QSAR. Fundamentals and Applications in Chemistry and Biology, American Chemical Society, Washington, DC, 1995.
[33] S. Griffin, S.G. Wyllie, J. Markham, J. Chromatogr. A 864 (1999) 221–228.
[34] C. Yamagami, K. Araki, K. Ohnishi, K. Hanasato, H. Inaba, M. Aono, A. Ohta, J. Pharm. Sci. 88 (1999) 1299–1304.
[35] D. Pissinis, L.E. Sereno, J.M. Marioli, J. Braz. Chem. Soc. 16 (2005) 1054–1060.
[36] K. Kulig, B. Malawska, Biomed. Chromatogr. 20 (2006) 1129–1135.