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pH-Partition profiles of 4-(3-oxo-1,2-benzisothiazolin-2-yl)phenyl and phenoxyalkanoic acids

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Abstract

The 1,2-benzisothiazolin-3-one nucleus is well known in the medicinal chemistry literature for the variety of biological effects exerted by its derivatives. In the present paper, the dependence of the *n*-octanol/buffer distribution coefficient (D) on pH of four 4-(3-oxo-1,2-benzisothiazolin-2-yl)phenyl and phenoxyalkanoic acids was investigated, employing the reference shake-flask method. From the analysis of the pH-partition profiles in the chosen partition system, the log P(AH), the log $P(A^-)$ and the pK_a values for each compound were determined. The physico-chemical data obtained were compared to the pK_a and log P values of the corresponding phenyl and phenoxyalkanoic acids, and an estimation of the lipophilic and electronic contribution of the 1,2-benzisothiazolin-3-one substituent in the *para*-position is proposed. The 1,2-benzisothiazolin-3-one nucleus behaves as a lipophilic, moderately electron-withdrawing group.

Keywords: Lipophilicity; pH-Partition profiles; 1,2-Benzisothiazolin-3-one; pK_a

1. Introduction

The 1,2-benzisothiazolin-3-one nucleus and related structures have extensively appeared in the literature over the years. 1,2-Benzisothiazolin-3-one proved to be a versatile moiety, being the central core in series of variously substituted derivatives endowed with a variety of biological effects. Derivatives presenting antimicrobial [1,2], genotoxic [3], antifungal [4], anti-inflammatory [5], and antiaggregating [6] activities have been described. The widespread use is also known of 1,2-benzisothiazolin-3-one and its derivatives as industrial microbiocides and preservatives; several investigations on the occupational allergies and dermatitis associated with its huge application in transformation industries have appeared in specialised literature [7–9].

A series of 4-(3-oxo-1,2-benzisothiazolin-2-yl)phenyl and phenoxyalkanoic derivatives, prepared by our group, showed anti-inflammatory and antimicrobial activity [1,5]. These compounds were selected for a QSAR study, where their antimicrobial potency, expressed as pIC_{50}, was correlated to the physico-chemical property lipophilicity, expressed by log $D_{7.0}$, by a bilinear relationship [1].

Given the importance, within this series of compounds, of the partition processes, which are in turn influenced by compound ionisation, we decided to further investigate their partition behaviour, focusing our attention on four representative acids. The aim of the work was also to evaluate the influence of a 1,2-benzisothiazolin-3-one group, introduced on a benzene ring, on the partition and ionisation of carboxylic acid derivatives.

Generally speaking, the partitioning of a single, non-ionisable molecular species in a biphasic system, under equilibrium conditions, is described by the partition coefficient (P), defined as the concentrations ratio of a single molecular species partitioning between two solvents and generally expressed in terms of its logarithm (log P) [10].

The *n*-octanol/water system remains the reference one for this type of measurement, although different organic

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solvents have been employed, in order to provide different kinds of information on the solute properties.

However, when a substance having ionisable functionalities is to be tested, a consideration of the partitioning of all the species, ionised and not, in the system is required: a redistribution of all the actual species in the two phases is observed, following pH variations. The lipophilicity parameter which takes into account the equilibrium of an ionisable compound, at a stated pH value, is the distribution coefficient (D), which depends on the P of the single, neutral species and on the pK_a values of the chosen compound [11].

Employing the standard shake-flask method and different n-octanol/buffer systems at different pH values, it is possible to obtain several one-point measurements by sampling the partitioning behaviour of the chosen compound over a large pH interval.

Buffer type and concentration, as well as ionic strength and temperature conditions, have to be considered among the determinants which can influence the final log D value [12]. The fitting of the experimental data with the theoretical equations relating log P, log D and pK_a values can help to obtain the dissociation constant values (pK_a) for the chosen compounds.

In the present work, we focused our attention on the measurements of distribution coefficients of four acid derivatives, representatives of the set of compounds that showed antimicrobial activity [1]. In particular, we selected a benzoic acid, a phenylacetic acid and two phenoxyalkanoic acid derivatives, to investigate the direct effect of the 1,2-benzisothiazolin-3-one group in a Hammett-like system, and to test the possible conjugation with the oxygen atom.

The log D values were measured over a wide range of pH values, in order to study their pH-partitioning profiles. Then, fitting the experimental results with the equation describing the case of the partitioning of a monoprotic acid, the apparent pK_a values were calculated. Finally, the log P, log D and pK_a values for the tested compounds were compared to the log P and pK_a values of the reference acids, to attempt an estimate of the lipophilic and electronic contribution of the 1,2-benzisothiazolin-3-one moiety to the whole molecule.

2. Experimental

2.1. Materials and reagents

The 1,2-benzisothiazolin-3-one derivatives used in this study were synthesised in our laboratories according to the procedures described in a previous article [5]. Benzoic, phenylacetic and 3-phenoxypropionic acids (>99% purity) were purchased from Sigma Aldrich Chemie, Germany. HPLC grade acetonitrile was obtained from BDH Laboratories Supplies, England. Phosphoric acid was purchased from Carlo Erba Reagents, Italy. Demineralised water was bidistilled before analysis. All buffers and n-octanol employed in the preparation of the partitioning systems were of analytical grade (Carlo Erba Reagents, Italy).

2.2. Shake-flask determination of log D values

The distribution coefficients (D) were determined at room temperature (r.t.) (21 ± 3 °C) by the shake-flask method. Briefly, the partition system consisted of mixtures of n-octanol and various buffered aqueous solutions [13]. The two phases were mutually saturated by stirring at r.t. for ca. 12 h. The ionic strength of the buffers was adjusted to 0.15 M with KCl. Small volumes of the compound solutions were shaken for at least 4 h, at r.t., with ca. 100 inversions of the vial per minute. Samples were then centrifuged at 3000 rpm for 10 min and the two phases were manually separated. Each was diluted with analytical grade methanol before injection in the HPLC apparatus; this avoided interference from the n-octanol or the buffer. The volume injected was 20 μl per run. All the runs were repeated at least four times. The D values were calculated from the ratio of the mean peak areas in the two phases, correcting for instrumental attenuation and dilution. The D values obtained were the means of at least four different determinations.

All the measurements were performed using an HPLC system equipped with a solvent delivery pump (Gilson 305 HPLC Pump), a 20-μl capacity sample injector (Rheodyne 7125 injector) and a UV detector (Gilson 115 UV Detector). The column was a Spherisorb ODS-2, 250×4.6 mm, 10 μm. The mobile phase consisted of acetonitrile and phosphoric acid 0.5% v/v in variable proportions at a flow rate of 1.0 ml/min, and the elution was monitored at 254 nm.

The mobile phase mixture was filtered through a 0.45-μm pore nylon membrane filter (Sigma Aldrich S.r.l., Italy). The peak areas were recorded using a stand-alone integrator (Hewlett Packard HP3394).

2.3. Analysis of the shake-flask pH-partition profiles

The experimental data were fitted with the theoretical equation describing the pH-partition behaviour in the biphasic system in the case of a monoprotic acid:

$$\log D_{pH} = \log P - \log[1 + 10^{(pH - pK_a)}] + \log[1 + 10^{(pH - pK_a - diff\ \log P)}] \quad (1)$$

with *diff* log P being the difference between the log P of the neutral and the ionised species, a parameter which takes into account the partitioning of the ion-pair [14].

The solver routine of EXCEL 97 (Microsoft Corp.) was employed in order to minimise the sum of squares of the differences between calculated and actual values. No

dependence of the experimental uncertainty on pH was observed.

3. Results and discussion

Table 1 reports the general structure of the compounds under investigation and the experimental distribution coefficients, expressed in terms of log D, measured over a wide range of pH values. The selected compounds are two phenyl and two phenoxyalkanoic acids, substituted in the *para*-position with the 1,2-benzisothiazolin-3-one moiety.

Figs. 1–4 show the four pH-partition profiles obtained by fitting the observed log D data with the theoretical equation (see Section 2).

It was not possible to measure the pH-partition profile of **1** beyond the pH value of 8.2 because of stability problems occurring at high pH values. However, the data allowed for the interpolation of the pK_a value.

In general, the four profiles present two plateau zones, one at low pH values, where, in the case of monoprotic acids, log D_{pH} should be equal to that of the unionised species, log P(AH), and another one, due to the partitioning of the ionised species, log $P(A^-)$, at high pH. In the central region, the log D value depends on the complex partition equilibrium of both the ionised and unionised species, and its value rapidly changes with pH variation. The first part of Table 2 shows the log P(AH) and log $P(A^-)$ values obtained by fitting the shake-flask one-point-measurements with the above mentioned equation.

In the second part of the table, the same physicochemical properties were determined experimentally for benzoic and phenylacetic acid. This enabled us to control the method's reliability through the correct superposition of the theoretical and experimental profiles, and to assess the accordance of the data with those

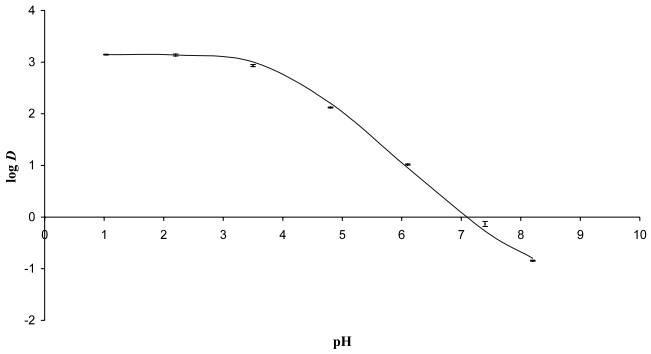


Fig. 1. pH-Partition profile for compounds **1**.

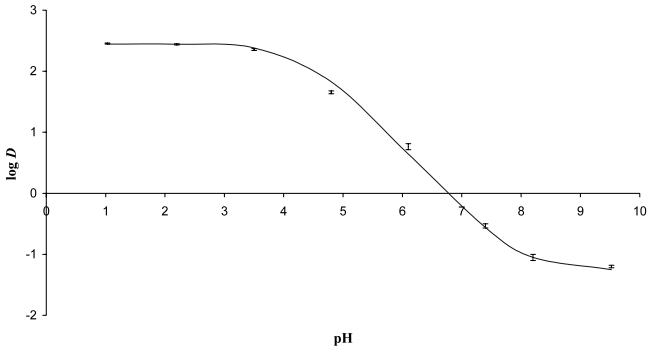
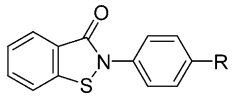


Fig. 2. pH-Partition profile for compounds **2**.

described in the literature [15]. For the phenoxyacetic acid, available literature data were considered [16], while the same experimental method was applied to 3-phenoxypropionic acid, as no literature data were found. C log P values are also presented in the table as terms of comparison; their accordance with experimental values is good. C log P proves adequate to describe the lipophilicity of this series of compounds.

The derived parameter *diff* log P_{N-I} [14] can be easily determined from the difference between the log P(AH) and log $P(A^-)$, log P of the neutral and of the ionised species, respectively. The parameter *diff* log P for a

Table 1
n-Octanol/buffer log D values at differing pH



Comp.	R	pH [a]							
		1.0	2.2	3.5	4.8	6.1	7.4	8.2	9.5
1	COOH	3.15(±0.01)	3.14(±0.02)	2.93(±0.02)	2.12(±0.01)	1.02(±0.01)	−0.13(±0.05)	−0.85(±0.01)	[b]
2	CH_2COOH	2.45(±0.01)	2.44(±0.01)	2.36(±0.02)	1.65(±0.02)	0.76(±0.05)	−0.53(±0.03)	−1.05(±0.05)	−1.20(±0.02)
3	OCH_2COOH	2.21(±0.02)	2.14(±0.01)	1.46(±0.04)	0.39(±0.02)	−0.58(±0.05)	−1.21(±0.01)	−1.28(±0.04)	−1.20(±0.01)
4	$O(CH_2)_2COOH$	2.65(±0.01)	2.63(±0.01)	2.57(±0.02)	2.00(±0.05)	1.03(±0.02)	−0.16(±0.02)	−0.92(±0.11)	−0.99(±0.01)

[a] For buffers used see Section 2. The means of at least four different determinations are represented (±SD).
[b] The compound was not stable enough to allow for measurement.

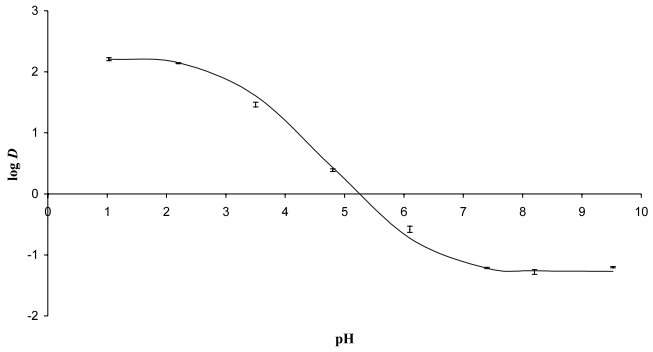


Fig. 3. pH-Partition profile for compounds **3**.

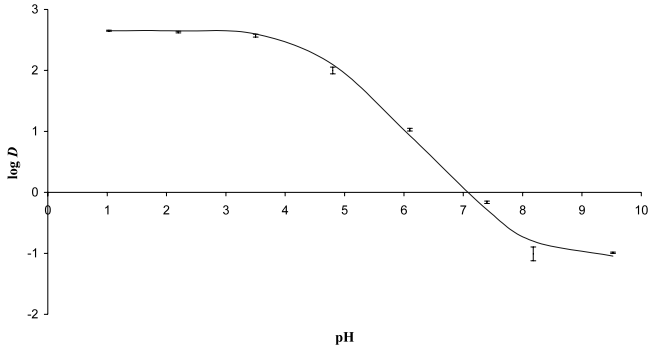


Fig. 4. pH-Partition profile for compounds **4**.

monoprotic molecule in the *n*-octanol/water system ranges from 3 to 4 [17]. Moreover, it has been shown that, within the same series of compounds, i.e. with related chemical structures, the *diff* log *P* remains rather constant [14]. This is applicable also to our case, where somewhat constant *diff* log *P* values were obtained, all falling in the range between 3.5 and 4.

Another column of Table 2 is dedicated to the evaluation of the π value for the 1,2-benzisothiazolin-3-one substituent in the four-position of the phenyl ring. The π value appears to depend on its environment, being lower in the series of the phenoxyalkanoic acids, probably owing to the perturbation of the electronic distribution of the phenoxy fragment caused by the 1,2-benzisothiazolin-3-one group. However, the observed π values were within the range 0.85–1.23.

In Table 2 the interpolated pK_a values, derived from the fitting of the shake-flask distribution curves, are also presented. The acidity of the derivatives increases with the order $O(CH_2)_2COOH < CH_2COOH < COOH < OCH_2COOH$, following the same trend as the reference acids. ΔpK_a values, being the difference between the pK_a of the tested compounds and of the reference acids, are also reported.

ΔpK_a could be explained by taking into account the dependence of pK_a on the electronic effect of the 1,2-benzisothiazolin-3-one substituent. For compound **1**, belonging to the series of four-substituted benzoic acids, the difference stabilises at 0.27, and equals its σ_p value [18]. For the other derivatives, the influence of the differing environment, described by different ρ values, has to be considered. In fact, the electronic effect has a weaker influence on the acid groups with higher topological distance. In the case of **2**, by employing the literature ρ value for the series of phenylacetic acids of 0.59 [18], a σ_p value of 0.32 was obtained, in accordance with the value obtained for **1**.

In conclusion, log *P* and pK_a values in the case of four 1,2-benzisothiazolin-3-one acid derivatives were obtained, with the shake-flask technique, by fitting the log *D* versus pH partition profiles. An estimate of the lipophilic and electronic contribution of the 1,2-benzisothiazolin-3-one nucleus is proposed. 1,2-Benzisothiazolin-3-one behaves as a weak electron-withdrawing, lipophilic substituent, having a π of 1.23 and a σ_p of 0.27 on the benzoic acid, and a weak dependence of its lipophilic and electronic contributions on the structure of the scaffold.

This result could be useful for QSAR studies including this interesting group as a substituent.

Table 2
Lipophilicity and pK_a values for derivatives **1**–**4** and corresponding reference acids

Comp.	log *P*(AH) [a]	log *P*(A^-) [a]	*C* log *P* [b]	$\pi_{benziso}$	pK_a [a]	ΔpK_a
1	3.15	[c]	3.10	1.23	3.91	0.27
2	2.45	−1.25	2.63	1.03	4.30	0.19
3	2.22	−1.28	2.56	0.87	3.02	0.15
4	2.65	−1.05	2.82	0.85	4.39	0.06
Benzoic acid	1.92	−0.83	1.89		4.18	
Phenylacetic acid	1.42	−1.11	1.41		4.49	
Phenoxyacetic acid	1.28 [d]	N.A.	1.35		3.17 [d]	
3-Phenoxypropionic acid	1.80	−1.68	1.61		4.45	

N.A., not available.
[a] Obtained by fitting the shake-flask pH-partition profile (see before).
[b] *C* log *P* version 4.73 (http://www.daylight.com).
[c] Stability problems at high pH did not allow for the determination.
[d] Literature value [16].

Acknowledgements

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