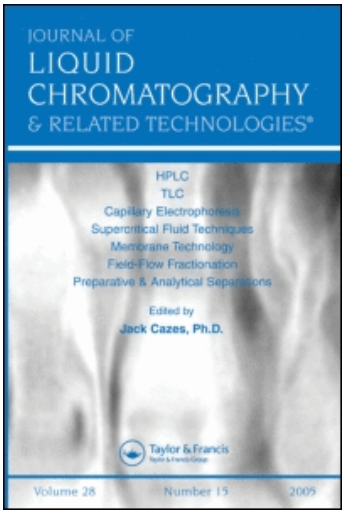

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 22 February 1999

J. LIQ. CHROM. & REL. TECHNOL., 22(3), 357–366 (1999)

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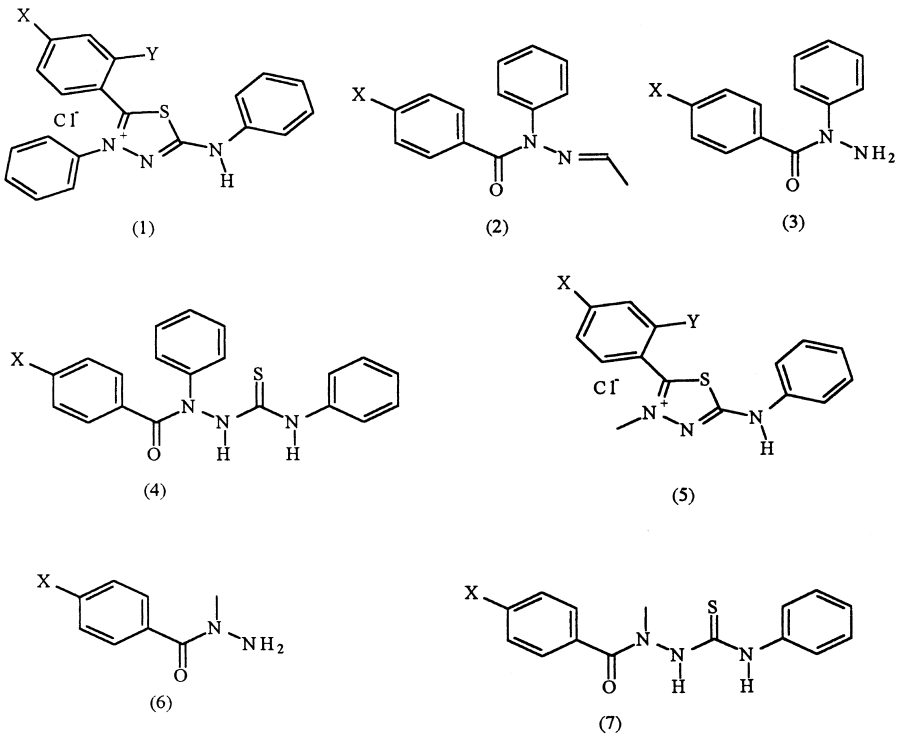
ABSTRACT

Mesoionic thiadiazoliumaminides, known generically as thioisosydnonimines, are antimicrobial drugs. Substitution of lipophilic substituents of mesoionic ring at 5-position increases their biological potencies against *Staphyloccocus aureus*, *Staphyloccocus epidermidis*, and *Bacilus cereus*. Mesoionic compound, which contains 2,4-Cl2-C6H3- moiety, is the most potent in the series. The lipophilicity measurement of log *P* using RP-HPLC for the newly synthesized intermediates and mesoionic compounds shows that these compounds are quite lipophilic and their behavior towards hydrogen bond capabilities do not allow any differentiation between H-donors, amphiprotics, and H-acceptors.



Y = H, X = (a) H, (b) Me, (c) MeO, (d) NO_2

Figure 1. Mesoionic 1,3,4-thiadiazolium-2-aminide conjugate acid and their precursors.

INTRODUCTION

Mesoionic compounds are very useful in medicinal chemistry due to their well-known range of pharmacological activities and the synthesis of new analogues in order to gauge their potential as chemotherapic agents is very important.[1] Recently, our group has synthesized mesoionics 1,3,4-oxadiazolium-2-aminide and 1,3,4-oxadiazolium-2-olate and evaluated their antimicrobial activity.[2] We have also studied the antimicrobial activity of mesoionic 1,3,4-thiadiazolium-2-aminides using the biological micro-calorimetric technique,[3] and elucidated their structures.[4-6] Mesoionic 1,3,4-thiadiazolium-2-aminides have also been assayed against some fungi and bacteria[7] and have shown some good activity against *Staphyloccocus aureus*, *Staphyloccocus epidermidis,* and *Bacilus cereus*. Although they have almost the same range of potencies, compound **1**(X = Y = Cl), Figure 1, is the most potent

one: MIC = 0.14 μM/mL.[7] It has seemed quite plausible to assume that the increase in lipophilicity of the lead compound **1**(X = Y = Cl) has lead to some qualitative relationship between chemical structure (i.e. physical and chemical properties) and biological potency.

The lipophilicity of a drug can be defined by the partition coefficient ***P***, which is the ratio of equilibrium concentrations of a drug in an organic phase and an aqueous phase: $P = C_{org}/C_{aq}$, and n-octanol/water has proven to be the system of choice for measuring partition coefficients for QSAR studies.[8] However, its experimental determination remains crucial. Out of dozens of methods that have been used to measure lipophilicity, the "shake-flask" method and reversed-phase high performance liquid chromatography, RP-HPLC, are, by far, the most popular ones.[9] Moreover, due to some problems associated with the "shake-flask" method the RP-HPLC is an encouraging tool.[10,11] Nevertheless, to measure the lipophilicity of a solute, the partition system employed has to be well assembled. Concerning to the RP-HPLC measurement, the partitioning system has to mimic a common lipophilicity reference scale which is provided by octanol-water partition system.[12]

It has been thoroughly shown that there is no universal defined hydrophobicity scale. Thus, there is always the need of determining a scale *priori* to obtaining log *P*s. The C-18 stationary phase is quite common (though many others are available),[9] but the mobile phase is still a matter of dispute,[13] although some recommendations lead to methanol/water.[9] Moreover, the chromatographic descriptor log k_w, that is the log k extrapolated to zero organic modifier content in the mobile phase, seems to be a reasonable chromatographic parameter.[11,14]

As far as QSAR and QSRR are concerned, the chromatographic data obtained are useful database for the future development of any kind of relationship. The development of a useful scale for the determination of log *P* for diverse chemical structures relies upon the application of their partition coefficients not only as "measured" values, but also to describe the utility of the chromatographic method in determining them. Therefore, this paper addresses the determination of a chromatographic scale via RP-HPLC in order to get the mesoionic log *P*s and to apply them to extend our understanding of such compounds properties.

EXPERIMENTAL

The RP-HPLC experiments were recorded on a Shimadzu instrument equipped with two bombs LC-10AD, UV detector SPD-6AV and LC-R6A.

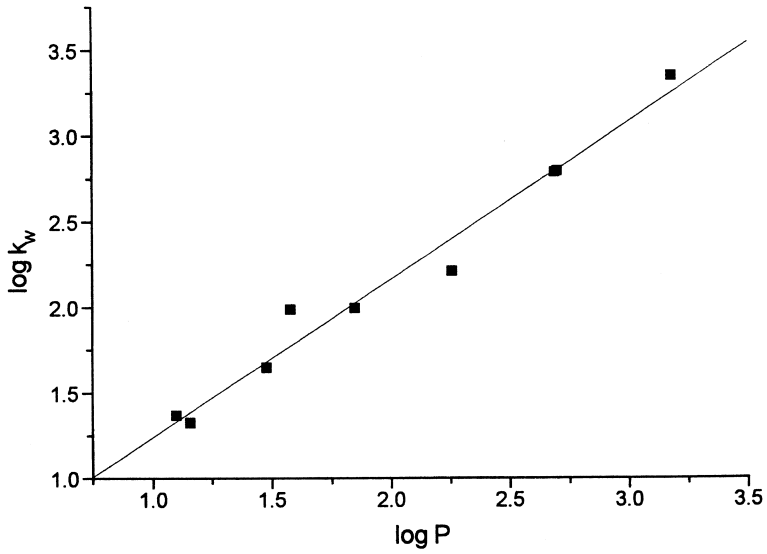


Figure 2. Linear dependence of log k_w versus log *P*.

The stationary phase was C_{18} ODS-Shim-Pack column (18.0 x 6.0 mm). The mobile phase was a buffer of 5.10^{-3} M of phosphoric and glacial acetic acids at pH 4.6 and methanol as modifier agent. The methanol content in the mobile phase composition (φ_{MeOH}) was in the range of 25/85 (% v/v).

RESULTS AND DISCUSSION

The set of compounds studied included mesoionic 1,3,4-thiadiazolium-2-aminides and their phenyl-hydrazone, phenyl-acylhydrazine and acyl-thiosemicarbazide precursors whose synthesis are discussed elsewhere.[15] These mesoionic compounds have no simple electronic structure and also are charged molecules, though neutral. The use of a single C_{18} column has proven to be very reproducible for neutral species as well as to validate the oddity of mesoionic log P measurements. The comparison between them allows one to validate these more complex structures and their charge effects, as well as their implicit diversity. If these are in agreement with the octanol-water partition coefficients, then the problem of measuring log *P* for so highly complicated mesoionic structures is readily solved and their RP-HPLC chromatographic values could be used in QSAR studies.

Equation 1 shows the standards (depicted in Figure 3) as measured for methanol/water content that is needed in order to derive log k_w. The correlation between log k_w and log P_{oct} is shown in Figure 2.

Model for the standards:

$$\log k_w = 0.923(\pm 0.13)\log P_{oct} + 0.317(\pm 0.28) \quad (1)$$
$$(n = 9,\ r = 0.987,\ s = 0.119,\ F = 263,\ r^2_{cv} = 0.960)$$

Equation 1 can be regarded as a good Collander type equation[16] and then C_{18} column can be used for the measurement of log P_{oct}. Log k_w can, therefore, also be called a good descriptor for log P_{oct}. This is also due to the *homo-energetic* retention that occurs in the two phase systems.[17]

It has been suggested in the recent literature that log k_w is a better descriptor for log P_{oct} than log k,[14] though there is still some doubts about the errors it encompasses. However, a closer insight of this latter chromatographic descriptor reveals some interesting results.

It is clear from our results that as the MeOH content in the mobile phase increases the slope and intercepts diminish, leading to different mechanism of retention due to the higher presence of the modifier, which certainly causes the disruption of the quasi-crystalline water structure.[10]

It has also been suggested that the slope obtained for log k versus φ_{MeOH} (the methanol content of mobile phase), S, is a measure of lipophilicity.[9,18,19] The straight lines in the composite diagram found in Figure 3, for all standards, are nearly parallel, which supports the same mechanism of retention and the capability of S being responsible for the hydrogen bond activity (see below).[19]

However, ethyl acetate (B) and formamide (C) have been excluded from the model (showed in Equation 1) due to the fact that they represent not only a partitioning mechanism but also something else; i.e. they do encompass any other retention parameters (electrostatic, charge transfer, van der Waals, etc.). In other words, it seems that purely partitioning is not being experienced by these two standards.

The chromatographic retention mechanism is dependent, basically, on two components: the solute's size (measured by its volume or surface area) and its capacity of establishing hydrogen bond. It has been shown that the slope, S, versus log k_w can display differences in the capacity of forming hydrogen bonding for a set of compounds,[20] see Figure 4. (The straight lines are fictitious and serve only to show the general behavior of S dependence upon log k_w.) S is

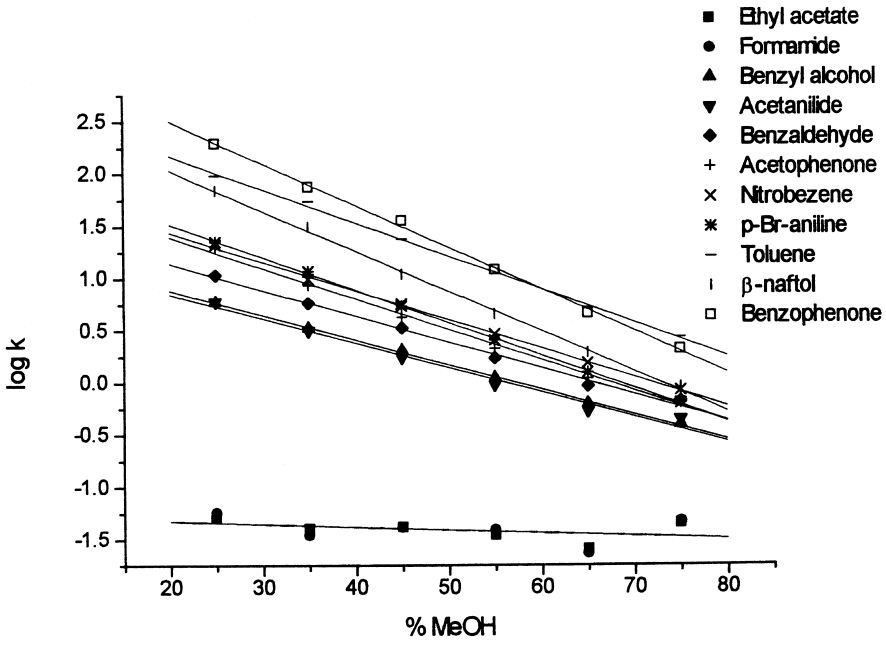


Figure 3. Composite diagram for the standards.

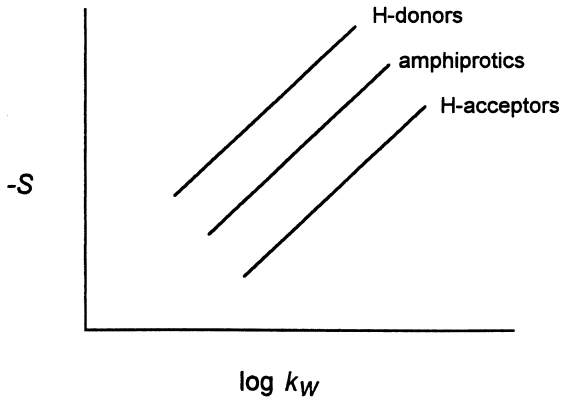


Figure 4. Parallel behavior for different kinds of chemicals according to their capability of forming hydrogen bonds. (Adapted from reference 20).

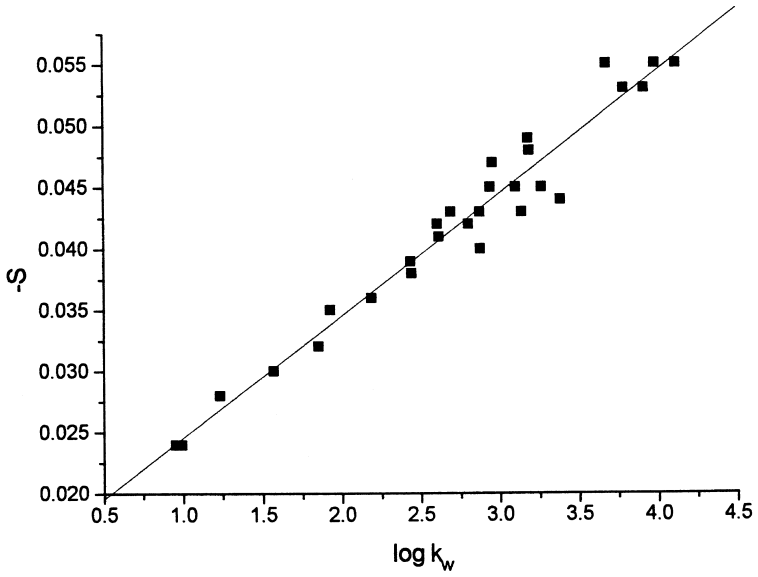


Figure 5. Linear dependence of S over log k_w, for the study compounds.

linearly correlated with log k_w (r = 0.925) and log P_{oct} (r = 0.920), for the standards. The slopes are 0.919 and 0.854, respectively. It is worthwhile to point out that for all of them there is a straight line; that is, they can be classified upon one class of hydrogen-bonding compounds.[21]

A plot of S versus log k_w, for the study compounds can be found in Figure 5, a straight line with a slope equal to 0.998. It means that all of them behave similarly to those standards and can, therefore, be evaluated in the same way. There is no clear separation between their capacity of forming hydrogen bonds. Thus, it seems obvious that for this descriptor they do have the same sort of retention to the chromatographic column used in this work.

The test set used in this work covers a broad range of lipophilicity from log P_{app} = 0.705 to 4.080, Table 1. The observed high correlation of log P_{oct} and log k_w (Equation 2) confirms the results and conclusions presented by Minick,[19, 22]. For this range of log P_{app} values there is no need for adding any OH-suppresser upon C_{18} column. Moreover, this method gives an insight of the log P_{app} for such odd mesoionic compounds and also for their corresponding precursors.

$$\text{Log } k_w = 0920(\pm 0.032) \log P + 0.319(\pm 0.088) \quad (2)$$
$$(n = 28,\ r = 0.966,\quad s = 0.075,\ F = 3595.56,\ r^2_{cv} = 0.992)$$

Table 1

Values of Log k_W and Log P_{app} for the Study Compounds

Compounds	Log K_W*	Log P_{app}
1a	2.958	2.844
1b	3.677	3.612
1c	3.190	3.081
1d	3.181	3.082
2a	2.880	2.761
2b	3.387	3.603
2c	3.268	3.175
2d	3.140	3.083
3a	2.444	2.295
3b	3.103	2.999
3c	2.942	2.827
3d	2.616	2.479
4a	3.785	3.728
4b	4.115	4.080
4c	3.982	3.938
4d	3.914	3.865
5a	1.927	1.743
5b	2.436	2.286
5c	2.187	2.020
5d	1.854	1.665
6a	0.956	0.705
6b	1.572	1.363
6c	1.233	1.001
6d	0.994	0.746
7a	2.605	2.467
7b	2.875	3.010
7c	2.803	2.561
7d	2.693	2.678

* $\text{Log } k_W = 0.920(\pm 0.032)\log P + 0.319(\pm 0.088)$.

Overall, log P_{app} for the mesoionic compounds cannot be easily calculated. Thus, the experimental determination through RP-HPLC represents a way of getting partition coefficients for such compounds. The method is quite reliable, straightforward, fast, and reproducible.

The RP-HPLC measurements have shown only one single peak for all compounds under study. This suggests that no ionisation has taken place and, even for the mesoionic compounds, the retention happens to follow an ion-pair movement (MI^+Cl^-). Our results extricate so far no differences in the retention mechanism due to hydrogen-bonding capacity of all study compounds, i.e., the conjugate mesoionic weak acids do behave likewise their neutral counterparts.

ACKNOWLEDGMENTS

We thank CNPq and FINEP for their grants, and one of us (M.M.B.) CNPq for their scholarship.

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Received January 27, 1998
Accepted April 28, 1998
Manuscript 4721

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