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Determination of partition coefficients n-octanol/water for treosulfan and its epoxy-transformers: An example of a negative correlation between lipophilicity of unionized compounds and their retention in reversed-phase chromatography

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ABSTRACT

For the last decade an alkylating agent treosulfan (TREO) has been successfully applied in clinical trials in conditioning prior to hematopoietic stem cell transplantation. Pharmacological activity of the pro-drug depends on its epoxy-transformers, monoepoxide (S,S-EBDM) and diepoxide (S,S-DEB), which are formed in a non-enzymatic consecutive reaction accompanied by a release of methanesulfonic acid. In the present study partition coefficient n-octanol/water (P_{OW}) of TREO as well as its biologically active epoxy-transformers was determined empirically (applying a classical shake-flask method) and *in silico* for the first time. *In vitro* the partition was investigated at 37 °C in the system composed of the pre-saturated n-octanol and 0.05 M acetate buffer pH 4.4 adjusted with sodium and potassium chloride to ionic strength of 0.16 M. Concentration of the analytes was quantified by reversed-phase high performance liquid chromatography (RP-HPLC) method in which retention time increased from S,S-DEB to TREO. It was shown that neither association nor dissociation of the tested compounds in the applied phases occurred. Calculated log P_{OW} (TREO: -1.58 ± 0.04, S,S-EBDM: -1.18 ± 0.02, S,S-DEB: -0.40 ± 0.03) indicate the hydrophilic character of the all three entities, corresponding to its pharmacokinetic parameters described in the literature. Experimentally determined log P_{OW} of the compounds were best comparable to the values predicted by algorithm ALOGPs. Interestingly, the P_{OW} values determined *in vitro* as well as *in silico* were inversely correlated with the retention times observed in the endcapped RP-HPLC column. It might be explained by the fact that a cleavage of methansulfonic acid from a small molecule of TREO generates significant changes in the molecular structure. Consequently, despite the common chemical origin, TREO, S,S-EBDM and S,S-DEB do not constitute a 'congeneric' series of compounds. We concluded that this might occur in other low-weight species, therefore measurement of their P_{OW} by RP-HPLC had to be applied with a special care.

1. Introduction

Clinical trial conducted in 2000 revealed that an anticancer drug treosulfan (TREO) used in conditioning prior to hematopoietic stem cell transplantation (HSCT) at single doses up to as much as 47 g/m^2 does not imply severe non-hematological toxicity [1]. Since that time TREO has been successfully applied as a myeloablative agent in pediatric and adult recipients prior to HSCT [2–5]. Currently, the drug has an orphan designation for this indication and the marketing authorization by the European Medicines Agency is expected in the near future [6,7]. Biological activity of the pro-drug TREO results from the fact that under physiological conditions of human blood it undergoes non-enzymatic conversion to S,S-EBDM and S,S-DEB, which are capable of alkylating DNA. The activation reaction is accompanied by a release of methanesulfonic acid (MA) (Fig. 1) [8,9]. Despite the mentioned facts, partition coefficient n-octanol/water (P_{OW}), a common quantitative descriptor of lipophilicity, has not been determined experimentally for TREO and S,S-EBDM. The available literature reports only P_{OW} of S,S-DEB at room temperature [10,11]. Meanwhile, it is generally known that lipophilicity is a crucial determinant of behavior of chemicals *in vivo*. The knowledge of this parameter contributes to understanding and interpretation of ADMET properties (absorption, distribution, metabolism, elimination and toxicity) as well as pharmacodynamic aspects of a drug action [12–14]. Therefore, in the present paper we describe experimental determination of P_{OW} for TREO and both its epoxy-transformers under *in vitro*

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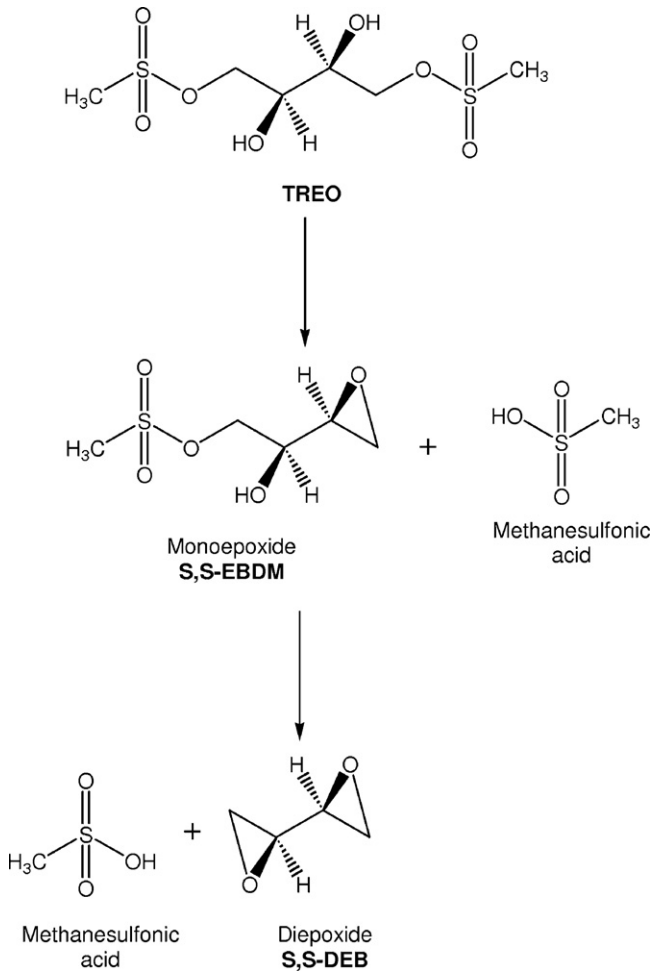

Fig. 1. TREO activation to biologically active epoxides.

conditions imitating human extracellular fluid and blood with respect to temperature and ionic strength. The obtained P_{OW} were discussed in view of the literature data regarding pharmacokinetics of the tested compounds. Interestingly, during the studies a negative relationship between the P_{OW} and retention time of the analytes in reversed-phase high performance liquid chromatography (RP-HPLC) was observed and this fact was discussed.

2. Material and methods

2.1. Chemicals and reagents

TREO was kindly donated by medac GmbH (Hamburg, Germany). Acetaminophen, (±)-1,3-butadiene diepoxide ((±)-1,2:3,4-diepoxybutane, (±)-DEB) and sodium acetate were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium hydroxide solution 0.1 M, sodium chloride, potassium chloride and acetic acid, all of analytical grade, were obtained from P.O.Ch. (Gliwice, Poland). n-Octanol, analytical grade, and acetonitrile, HPLC gradient grade, were purchased from Merck KGaA (Darmstadt, Germany). Demineralized water at a conductivity of 0.1 μS/cm, prepared in deionizer Simplicity UV (Millipore, MA, USA) and filtered through a 0.45 μm cellulose membrane filter (Sartorius, Germany), was always used.

2.2. Standard solutions of TREO and its epoxy-transformers

Stock solution of 20 mM TREO was prepared by dissolution of 0.0557 g of the compound in water and filling up to 10 mL in a volumetric flask. Stock solution of 20 mM (±)-DEB, which is an easily commercially available equimolar mixture of S,S-DEB and its enantiomer, was obtained by transferring 15.5 μL of the compound (at room temperature) into a 10 mL volumetric flask and filling up with water. Due to the fact that S,S-EBDM cannot be received from the commercial suppliers, the compound was obtained as a mixture with TREO and S,S-DEB by alkalization of TREO, similarly to procedure specified by Główka et al. [15]. Briefly, 0.0557 g of TREO was dissolved in 6 mL of water in a 10 mL volumetric flask, mixed with equimolar amount of 2 mL of 0.1 M NaOH and filled up with water to 10 mL. The obtained stock solution contained approximately 5 mM TREO, 10 mM S,S-EBDM and 5 mM S,S-DEB (molar ratio 1:2:1). Real concentrations of the compounds in the solution were established and amounted 5.66, 9.20 and 5.14 mM, respectively.

2.3. Acetate-buffered saline

Acetate-buffered saline concentrate was prepared as a mixture of 274 mM sodium chloride, 5.4 mM potassium chloride and 100 mM acetic acid–sodium acetate buffer adjusted to pH 4.4 with 1 M sodium hydroxide. Acetate-buffered saline for investigation of P_{OW} was prepared from the above concentrate by dilution with water (1:1, v/v). Ionic strength of the obtained solution (160 mM) and concentrations of sodium chloride (137 mM) and potassium chloride (2.7 mM) were patterned on a typical phosphate buffered saline to correspond approximately to physiological conditions of human blood and extracellular liquid.

2.4. Preliminary estimation of log P_{OW}, pK_a and surface area from molecular structure

Theoretical values of log P_{OW} for TREO and its epoxy-transformers were calculated using various substructure and whole molecule-based methods provided by ALOGPs 2.1 application, available at http://www.vcclab.org/lab/alogps. Additionally, values of pK_a and molecular surface area for the studied compounds were estimated using Marvin Sketch 5.10.2 software (ChemAxon Ltd.) available at http://www.chemaxon.com/marvin/sketch/index.php.

2.5. Shake flask method

P_{OW} for TREO and its epoxy-transformers was determined using a classical shake-flask method. The procedure was mainly based on the current guidelines of the Organization for Economic Cooperation and Development (OECD) [16]. An aqueous phase of the system constituted the acetate-buffered saline at pH 4.4, which was necessary for halting pH-dependent activation of TREO. As a non-polar phase, n-octanol pre-saturated with the acetate-buffered saline was used. Determination of P_{OW} was carried out at 37 °C.

2.5.1. Preparation of n-octanol/saline system

Pre-saturation of n-octanol was achieved by shaking a mixture of n-octanol and the acetate-buffered saline for 24 h at 37 °C. The aqueous phase of the system, containing TREO and its epoxy-transformers, was prepared by mixing the standard solution of TREO, S,S-EBDM and S,S-DEB, alone TREO or alone (±)-DEB with the acetate-buffered saline concentrate (1:1, v/v). In this manner, a two-fold diluted solution of the studied compounds in the acetate-buffered saline was obtained. Thereafter, an appropriate volume of the prepared aqueous phase was transferred into 24-mL glass vials and mixed with 20 mL of n-octanol pre-saturated with the acetate-buffered saline. The tightly closed vials were completely immersed

Table 1
Composition of n-octanol/acetate buffered saline systems studied in runs I–VI.

Run (n = 5)	n-Octanol: saline volumes (mL:mL)	Initial nominal concentration in the saline [μM]		
		TREO	S,S-EBDM	S,S-DEB
I	20.0:1.0	2828.4	4601.8	2569.9
II	20.0:0.5	2828.4	4601.8	2569.9
III	20.0:2.0	2828.4	4601.8	2569.9
IV	20.0:1.0	1414.2	2300.9	1284.9
V	20.0:1.0	2500.0	–	–
VI	20.0:1.0	–	–	2500.0

in a horizontal position in a water bath set at 37 °C and shook with a mechanical shaker (100 cycles/min, amplitude 9 cm) over 2 h. Preliminary studies demonstrated that under the above conditions, partition equilibrium was achieved and the studied compounds were chemically stable in the both phases.

The P_{OW} was determined in 6 different runs differing in a volume ratio of the phases and an initial concentration of the compounds in the aqueous phase (Table 1). Such a procedure was aimed to experimentally verify possible deviations from the Nernst partition law due to dissociation or association of TREO and its epoxy-transformers in the n-octanol/saline system. For a first run, a volume ratio of n-octanol to the saline was equal to 20:1. For a second run, the original volume ratio was multiplied by two and, for a third run, it was divided by two. In the fourth run, the initial quantity of the compounds in the saline was two-fold decreased. Additionally, in order to confirm that the simultaneous presence of the three analytes in the system (in runs I–IV) did not affect the results of P_{OW} for the particular compounds, two runs were performed in which solutions of alone TREO (run V) and alone (±)-DEB (run VI) were applied. In all the runs, five samples were prepared and processed at the same time.

2.5.2. Chromatographic analysis of TREO and its epoxy-transformers

When the shaking process was finished, vials containing n-octanol and the acetate-buffered saline were immediately centrifuged at 1620 × *g* over 5 min in order to achieve better separation of the phases. Upper n-octanol layer was carefully removed with a pipette and the exposed saline layer was sampled. Concentrations of TREO and its epoxy-transformers in the obtained aqueous phase were determined using a validated HPLC method with refractometric detection [15], after a slight modification. Briefly, a volume of 250 μL of the aqueous phase containing the analytes was mixed with 250 μL of water and 25 μL of acetaminophen (internal standard, IS) solution and then 100 μL of the resulting mixture was injected twice into the Agilent 1200 HPLC system with refractometric detector (Agilent Technologies, Waldbronn, Germany). Calibration standards were prepared by spiking 250 μL of the acetate-buffered saline with 250 μL of the standard solution of TREO, S,S-EBDM and S,S-DEB and 25 μL of IS. Separation of the analytes was performed in octadecylsilane (ODS) Hypersil column (150 mm × 4.6 mm, 5 μm), from Agilent Technologies, at 25 °C with a 1 mL/min rate flow of the mobile phase containing 0.01 M sodium acetate–acetic acid buffer pH 4.5 and acetonitrile (95:5, v/v). Typical chromatogram obtained during the analysis is presented in Fig. 2.

2.5.3. Calculation of P_{OW} and statistics

Initial concentrations of TREO and its epoxy-transformers in the aqueous phase (before mixing with n-octanol) and equilibrium concentrations of the compounds after the inter-phase partition, were calculated as a mean from two injections of each study sample, using the appropriate equations of the calibration curves. P_{OW} values were calculated according to the formula:

$$P_{OW} = \frac{C_O - C_{aq}}{C_{aq}} \times \frac{V_{aq}}{V_{oct}}$$

where C_o and C_{aq} denote the initial and equilibrium concentration of the compound in the aqueous phase, respectively; V_{aq} and V_{oct} denote volumes of the aqueous and n-octanol phases, respectively. Mean and SD of P_{OW} of the compounds obtained in the particular run I–VI (n = 5) as well as in all the runs together (n = 25 for TREO and S,S-DEB, n = 20 for S,S-EBDM) were calculated in Microsoft Excel 2007. Additionally, log P_{OW} and its SD were also calculated according to the following formulae:

$$\text{Mean}_{\log P_{OW}} = \log(\text{mean}_{P_{OW}})$$

$$\text{SD}_{\log P_{OW}} = \frac{1}{\ln 10 \times \text{mean}_{P_{OW}}} \text{SD}_{P_{OW}}$$

The obtained results of P_{OW} were subjected to a statistical analysis in Statistica 10 (StatSoft, Inc.). Normal distribution of the P_{OW} values within each run I–VI was examined using Shapiro–Wilk test, whereas homogeneity of variances between the runs was confirmed by Brown–Forsythe test. Moreover, for each compound, statistical significance of the differences between the mean P_{OW} values obtained in the particular runs I–VI was examined with one-way ANOVA (provided that normal distribution and homogeneity of variances were stated). All the tests were carried out at significance level α = 0.05 and a probability (p) value < 0.05 indicated statistical significance.

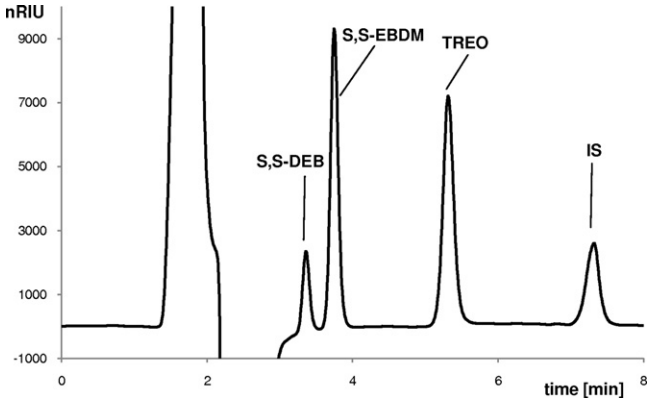

Fig. 2. HPLC chromatogram obtained during analysis of the acetate-buffered saline spiked with calibration standard of TREO (1131 μM), S,S-EBDM (1841 μM) and S,S-DEB (1028 μM).

3. Results and discussion

3.1. Computed values of log P_{OW}

Preliminarily estimated values of log P_{OW} for TREO and its transformers, ranging from −3.35 to −0.02 (Table 2), indicated the hydrophilic character of the tested compounds. Such results supported the choice of a shake-flask method for experimental study of P_{OW}. According to the OECD recommendations, this method is appropriate for determination of log P_{OW} ranging from −2 to 4, while an HPLC method can be applied when log P_{OW} spans from 0 to 6 [16,17].

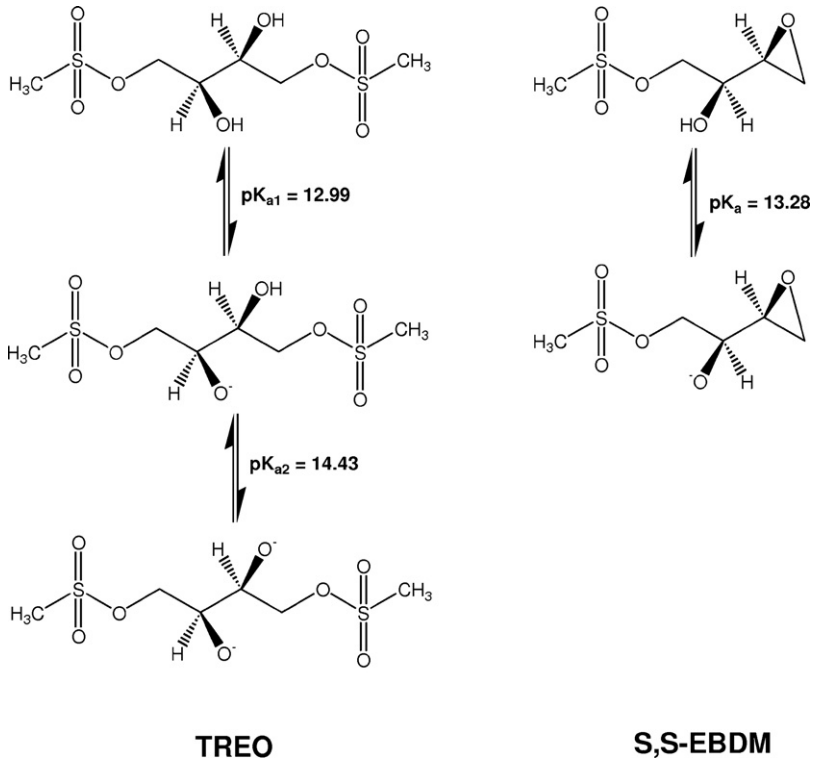

Fig. 3. Neutral and ionized form of TREO and S,S-EBDM and the corresponding values of pK$_a$, generated with MarvinSketch software.

3.2. Determination of pK_a

Structures of non-ionized and ionized forms of TREO and S,S-EBDM as well as corresponding values of pK$_a$ (Fig. 3) generated with Marvin Sketch software demonstrate these two compounds are theoretically capable of ionization due to deprotonation of the alcohol group. However, they are very weak acids, which is in accordance with known low acidity of aliphatic alcohols (for instance, pK$_a$ for methanol is equal to 15.2). Opposite to the above compounds, S,S-DEB does not undergo ionization at all, due to a lack of acidic hydrogen in its molecule. The obtained results indicated that during determination of P_{OW} with the shake-flask method, in the aqueous phase at pH 4.4, TREO and its epoxides were supposed to exist only in their non-ionized forms as required by the Nernst partition law. Noteworthy, also under physiological conditions the species should behave likewise. It excludes the possibility of their ion-pair interactions with plasma proteins and the tissue constituents *in vivo* [13,14].

Table 2
Theoretical values of log P_{OW} for TREO, S,S-EBDM and S,S-DEB computed from the molecular structure.

Method	TREO	S,S-EBDM	S,S-DEB
Atom-based methods			
ALOGP	−1.95	−1.17	−0.39
XLOGP2	−2.33	−1.42	–
XLOGP3	−2.16	−1.28	−0.52
Fragmental methods			
KOWWIN	−3.35	−1.32	−0.58
AB/LogP	−2.00	−1.93	−0.38
miLog *P*	−2.26	−1.14	−0.02
Topological descriptors-based methods			
ALOGPs	−1.53	−0.91	−0.36
AC logP	−2.35	−1.34	−0.32
MLOGP	−2.01	−1.53	−0.47

3.3. Experimentally determined P_{OW} and its relation to pharmacokinetics

Epoxides are quite reactive species, therefore stability of S,S-EBDM and S,S-DEB in n-octanol/acetate-buffered saline system at 37 °C was confirmed in the preliminary studies. After 2 h, which were sufficient for achievement of the partition equilibrium of TREO as well as its epoxy-transformers, no decrease in concentration of the compounds occurred in the sampled aqueous phase of the system within the next 4 h. Those results are in accordance with the stability of epoxybutene in saline/air and oil/air systems during 3 h shaking at 37 °C and a lack of a non-enzymatic decomposition of epoxybutene and DEB (stereochemistry not specified) in the muscle homogenate/air and muscle homogenate/hexane systems, respectively [18,19]. Results of experimental determinations of P_{OW} for TREO, S,S-EBDM and S,S-DEB are presented in Table 3. All values of log P_{OW} of the particular compounds fell within a range of ±0.22 units, complying the OECD acceptance criterion for precision of the measurements (up to ±0.3) [16]. The statistical analysis demonstrated that within all the runs I–VI, P_{OW} values of TREO and its epoxides were normally distributed ($p > 0.05$ in Shapiro–Wilk test). Moreover, the variances of P_{OW} obtained for each compound in the different runs were homogenous as proved by Brown–Forsythe test ($p > 0.05$). ANOVA test demonstrated a lack of statistically significant differences between the mean P_{OW} values obtained for each individual compound in the runs I–VI. Obtainment of the comparable P_{OW} for the various n-octanol/saline volume ratios (runs I–III) and different quantities of the tested substances in the system (runs I and IV) confirmed that neither association of the molecules in the octanol phase, nor their dissociation in the aqueous phase occurred. Furthermore, a lack of the P_{OW} differences between the systems in which a mixture of TREO, S,S-EBDM and S,S-DEB was present (runs I–IV) and the systems containing alone TREO (run V) or alone (±)-DEB (run VI) confirmed that the presence of the other analytes did not affect determination of P_{OW} for the individual analyte.

Table 3
P_{OW} and corresponding log P_{OW} values for TREO and its epoxy-transformers (mean ± SD, for each run $n = 5$) obtained in n-octanol/acetate buffered saline system at 37 °C.

Run	TREO		S,S-EBDM		S,S-DEB	
	P_{OW}	log P_{OW}	P_{OW}	log P_{OW}	P_{OW}	log P_{OW}
I	0.0260 ± 0.0032	−1.58 ± 0.05	0.0661 ± 0.0046	−1.18 ± 0.03	0.381 ± 0.012	−0.42 ± 0.01
II	0.0278 ± 0.0033	−1.56 ± 0.05	0.0656 ± 0.0042	−1.18 ± 0.03	0.401 ± 0.026	−0.40 ± 0.03
III	0.0250 ± 0.0032	−1.60 ± 0.06	0.0659 ± 0.0040	−1.18 ± 0.03	0.395 ± 0.014	−0.40 ± 0.02
IV	0.0267 ± 0.0010	−1.57 ± 0.02	0.0645 ± 0.0015	−1.19 ± 0.01	0.405 ± 0.040	−0.39 ± 0.04
V	0.0264 ± 0.0013	−1.58 ± 0.02	–	–	–	–
VI	–	–	–	–	0.424 ± 0.024	−0.37 ± 0.02
Total	0.0264 ± 0.0026	−1.58 ± 0.04	0.0655 ± 0.0035	−1.18 ± 0.02	0.401 ± 0.027	−0.40 ± 0.03

Eventually, P_{OW} values for TREO and its epoxy-transformers were calculated as a mean from all the conducted runs. Corresponding log P_{OW} values amounted to −1.58 ± 0.04 for TREO, −1.18 ± 0.02 for S,S-EBDM and −0.40 ± 0.03 for S,S-DEB. Experimental log P_{OW} for DEB (stereochemistry not specified) measured so far by Hansch et al. [11] and Deneer et al. [10] in n-octanol/pure water system at 25 °C were equal to −0.52 and −0.29 ± 0.19, respectively. Although experimentally determined values of P_{OW} for neither TREO nor S,S-EBDM have been yet published, the relevant data are available for busulfan (log P_{OW} −0.58, according to [20]), of which TREO is a structural analog. Comparison of log P_{OW} for these two structurally similar compounds proves that TREO is more hydrophilic which is in accordance with two additional hydroxyl groups in TREO molecule.

Obtained experimental values of log P_{OW} (Table 3) were compared to the theoretical results computed from the molecular structure (Table 2). Among nine methods applied to calculation of log P_{OW}, there was at least one algorithm for each individual compound which predicted log P_{OW} with high accuracy. Regarding S,S-EBDM and S,S-DEB it was ALOGP (−1.18 *vs.* −1.17 and −0.40 *vs.* −0.39, respectively), while in case of TREO the most accurate results were generated by ALOGPs (−1.58 *vs.* −1.53). Moreover, only ALOGPs, which is based on topological descriptors, provided acceptable closeness (±0.3 units) of the theoretical to experimental values simultaneously for all the studied compounds. The other algorithms produced accurate results only for the two epoxy-transformers (ALOGP, XLOGP3 and KOWWIN) or one of them, but not for TREO, which probably results from a general rule that accuracy of log P_{OW} prediction decreases with an increasing number of nonhydrogen atoms [21]. The similar results have been obtained by Mannhold et al. [22], who tested validity and accuracy of different algorithms on over 96,000 compounds. ALOGPs, in particular, and ALOGP as well as XLOGP3 belonged to those which provided the highest correlation between experimental and theoretically predicted values of log P_{OW}. So was miLogP, however in our research log P_{OW} produced by this algorithm was consistent with the experimental value only for S,S-EBDM.

High hydrophilicity of TREO (log P_{OW} −1.58 ± 0.04) is reflected in the literature data on the drug pharmacokinetic parameters, *inter alia* relatively low volume of distribution. Its value is equal to 20–34 L in adults [1,23,24] and 7–37 L in children [25]. Besides lipophilicity of a drug, also its charge and plasma protein binding govern blood and tissue affinity and, thus, are important determinants of volume of distribution [13,14]. As proved in Sections 3.2 and 3.3, TREO practically does not undergo ionization and exists only as the uncharged entity. Separate studies of TREO binding to proteins have not been carried out, however, based on almost complete recovery (96 ± 4%) of the drug from plasma after ultrafiltration (cut-off 10,000), it could be easily concluded that it practically does not bind to plasma proteins [24]. Actually, this stems logically from low lipophilicity of TREO and its neutral character, which both prevent hydrophobic and ion-pair interactions – two major forces in drugs binding to proteins [13,14]. All the above facts gathered together indicate that TREO undergoes distribution from a systemic circulation to aqueous compartments of the body but, in opposition to lipophilic drugs in unionized form, does not accumulate in tissues due to protein binding. Hydrophylic character of TREO corresponds also to a lack of its first-pass metabolism and preferable excretion of the unchanged drug by the renal route [1,13,14,23–26]. In turn, low lipophilicity of S,S-EBDM as well as S,S-DEB is reflected in the literature data on their low binding (∼20%) to human plasma proteins [27]. So far results of pharmacokinetics of TREO epoxy-transformers in humans have not been published. However, relevant data exist with reference to intravenous bolus of alone (±)-DEB in rats. Similarly to TREO, volume of distribution of (±)-DEB was relatively small (0.73 L/kg), which, according to the authors, resulted mainly from high water solubility of the compound [28].

Based on the obtained experimental log P_{OW} and molecular structure of TREO and its epoxy-transformers, it could be stated that all the three compounds satisfy all criteria of the Lipinski rule of five, this is: log $P_{OW} < 5$, molecular mass <500, number of hydrogen bond donors (sum of OH and NH) <5, and number of hydrogen bond acceptors (sum of O and N) <10. Consequently, they are likely to demonstrate good gastro-intestinal absorption and permeation across the biological membranes [12]. Indeed, available pharmacokinetic data on TREO and (±)-DEB confirm the above thesis. Clinical trials demonstrated practically 100% bioavailability of TREO after oral administration and its fast distribution characterized by $t_{0.5\alpha}$ 12 min [24,26]. Taking into account low P_{OW} of TREO, other factors such as a small molecular size, molecular surface properties and paracellular passage through aqueous pores appear to be engaged into efficient transfer of the drug across gastrointestinal tract and other membranes, including bone marrow. The same explanation probably applies to (±)-DEB, of which $t_{0.5\alpha}$ value observed in rats after the intravenous administration was as short as 2.7 min [13,14,28].

3.4. Inverse correlation between P_{OW} and retention in HPLC

Negative values of log P_{OW} of TREO and its epoxy-transformers proved hydrophilicity of all these compounds, with the parent drug being the most hydrophilic. It is here noteworthy that in the HPLC method [15] applied for simultaneous determination of TREO, S,S-EBDM and S,S-DEB in the present study, the parent drug had the longest retention time on the ODS column with a hydrophilic mobile phase containing only 5% (v/v) of an organic modifier (acetonitrile), while S,S-DEB had the shorter one (Fig. 2). Following the change of the buffer/acetonitrile ratio in the mobile phase, all the studied compounds exhibited a typical behavior as their retention times increased when acetonitrile percentage was lowered to 3% and decreased when the organic modifier content was 7%. All the time the order of elution was totally inversed compared to that expected on the basis of the determined P_{OW}. On the other hand, a positive correlation was observed between the retention of TREO, S,S-EBDM and S,S-DEB and their molecular surface (523, 383 and

246 Å^2, respectively) as well as a number of hydroxyl groups in their molecules. It might raise the hypothesis that TREO molecules were the most retained because they could develop the stronger attraction with ODS due to the largest molecular area or, alternatively, they experienced hydrogen bonding interaction with the residual silanol groups. It is a generally known rule that a positive relationship between the retention time in RP-HPLC and P_{OW} (preferably in the form of linear plot of log P_{OW} against logarithm of the retention factor) can be easily found for structurally related compounds (so called congeneric effect). The most common example is a series of n-alkanes, where addition of the methylene group results in increased lipophilicity, increased molecular surface and longer retention in the ODS column [17,29]. In opposition, although S,S-EBDM and S,S-DEB originate from TREO after release of MA, these three compounds cannot be considered as structurally related entities. It stems from the fact that they are small molecules and merely the cleavage of MA produces relatively significant changes in their structure, mainly appearance of an oxirane ring instead of more hydrophilic hydroxyl group. Logically, a similar situation in which even a slight structural difference generates a relatively considerable change in the physical properties is particularly possible for other low-weight compounds. In such cases a misusing of HPLC method for indirect determination of P_{OW} seems to be the most probable. If this method is anyway applied, a special care should be taken when selecting reference substances for preparation of the log P_{OW} – log(retention factor) calibration curve.

4. Conclusion

The results presented in this paper constitute the first report on experimental P_{OW} of TREO and S,S-EBDM. Determined values of P_{OW} demonstrate the hydrophilic character of TREO as well as its epoxy-transformers and correspond to their pharmacokinetic parameters. From a chromatographic point of view, TREO, S,S-EBDM and S,S-DEB do not constitute a congeneric series of compounds, therefore their retention in RP-HPLC does not correlate with the lipophilicity expressed by P_{OW}.

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