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RESEARCH ARTICLE

LIPOPHILICITY OF SOME 17-HALOGEN-16, 17-SECOESTRA-1, 3, 5 (10)-TRIENE DERIVATIVES

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ABSTRACT

Retention behaviour of two pairs of 17-halogen-16,17-secoestra-1,3,5(10)-triene derivatives in RPLC was studied. Commercial column 5μm- Spherisorb ODS-2, 124 × 4 mm was used. Mobile phase was acetonitrile-water in various proportions. Flow rate of mobile phase and column temperature varied. Correlation among various chromatographically obtained hydrophobicity parameters (log k_{to} and log k_{fro}) of 17-halogen-16,17-secoestra-1,3,5(10)-triene derivatives with log *P*, calculated by using commercial program ACD log *P* software (Advanced Chemistry, Toronto, Canada) was examined. The log k_{to} and log k_{fro} were obtained by linear extrapolation of retention of two pairs of 17-halogen-16,17-secoestra-1,3,5 (10)-triene derivatives to 0 of column temperature and to 0 of flow rate of the mobile phase.

INTRODUCTION

Steroids represent a great field of interest due to their various biological activities. One type of a biological activity can be changed by introducing and/or changing hydrophilic and hydrophobic functional groups, as well as by transforming the skeleton of a steroid molecule. Steroids are interesting for chromatographic research as they create a possibility for studying effects of substituent's on retention. Work on the steroid molecule was continued with the synthesis of one set of estrogen derivatives in order to functionalize rings A and D of the skeleton, and, consequently, an attempt was made to change the hormonal activity of estrone (Jovanović-Šanta et al., 2000). Estrogen hormones are female hormones and they promote both primary and secondary female characteristics. They play an important role in functioning of the brain, bones, liver, and cardiovascular system (Palomino, 1997). The hydrophobic nature of chemical agents often seems to be the most important physicochemical factor which determines their biological activity. The partition coefficient, *P*, of a compound between nonaqueous and aqueous phases can be used as a measure of its hydrophobicity. The traditional experimental method for the determination of log $P_{o/w}$, is the *shake flask* method (www.epa.). Nowadays liquid chromatography has a tendency to replace the tedious and poor interlaboratory reproducible *shake flask* method for measuring partition coefficients. Among liquid chromatography methods, RPLC is an alternative technique that can correlate the hydrophobicity of compounds with the retention parameters (Testa *et al.,* 2006; Pliška *et al.,* 1996; Djaković-Sekulić *et al.,* 2002). For purpose of avoiding practical difficulties that often arise in the direct determination of the partition coefficient, extrapolated retention parameters of various organic-water eluent compositions were used for measuring hydrophobicity. The intercept log k_0 corresponds to the retention in water as a mobile phase, and represents the commonly employed chromatographic hydrophobicity parameter. In addition to the experimental method, a number of other methods for the calculation of 1-octanol-water partition coefficients have been established (Todeschini and Consonni, 2002).

The aim of this work was to determine the retention constants of 17-halogen-16,17-secoestra-1,3,5 (10)-triene derivatives (log k_{to} and log k_{fro}) and to correlate these constants with log *P* calculated by the fragmental based method using the ACD log *P* software (Advanced Chemistry, Toronto, Canada) (Kaliszan, 2007; Heberger 2007). The log k_{to} and log k_{fro} were obtained by linear extrapolation of retention of 17-halogen-16, 17-secoestra-1,3,5 (10)-triene derivatives to 0 of column temperature and to 0 of flow rate.

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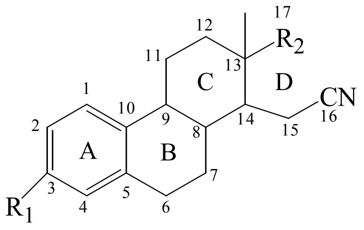
The compounds and their structures are listed in Figure 1.



Compound	R_1	R_2	log P
1	OH	CH_2F	3,90
2	$OCH_2C_6H_5$	CH_2F	6,21
3	OH	CH_2Cl	4,36
4	$OCH_2C_6H_5$	CH_2Cl	6,67
5	OH	CH_2Br	4,54
6	$OCH_2C_6H_5$	CH_2Br	6,84
7	OH	CH_2I	4,89
8	$OCH_2C_6H_5$	CH_2I	7,19

IUPAC names of steroids:

1. 3-Hydroxy-17-fluoro-16,17-secoestra-1,3,5(10)-triene-16-nitrile
2. 3-Benzyloxy-17-fluoro-16,17-secoestra-1,3,5(10)-triene-16-nitrile
3. 3-Hydroxy-17-chloro-16,17-secoestra-1,3,5(10)-triene-16-nitrile
4. 3-Benzyloxy-17-chloro-16,17-secoestra-1,3,5(10)-triene-16-nitrile
5. 3-Hydroxy-17-bromo-16,17-secoestra-1,3,5(10)-triene-16-nitrile
6. 3-Benzyloxy-17-bromo-16,17-secoestra-1,3,5(10)-triene-16-nitrile
7. 3-Hydroxy-17-iodo-16,17-secoestra-1,3,5(10)-triene-16- nitrile
8. 3-Benzyloxy-17-iodo-16,17-secoestra-1,3,5(10)-triene-16-nitrile

Fig. 1. Chemical structures of the compounds studied. Values of log *P* of compounds 1-8 are presented in the last column of Table.

EXPERIMENTAL

HPLC separations on C-18 column were performed on an Agilent 1100 Series HPLC (Agilent Technologies, USA) including a Degasser G1379 A, binary G1312 pump, ALS G1313A, COLCOM G1316A and DAD G1315B. The column used was commercially available particle size Spherisorb ODS-2 5µm, 124 × 4 mm (Agilent). 17-halogen-16,17-secoestra-1,3,5 (10)-triene derivatives (Figure 1) were synthesized with original reactions or with the application of literature methods (Jovanović-Šanta *et al.,* 2000). Behaviour and separation of two mixtures: mixture I (compounds 1, 3, 5 and 7) and mixture II (compounds 2, 4, 6 and 8) were applied to the columns as 0.001% solutions in methanol and solutions filtered through a 0.2 µm Chromafil filter (Macherey-Nagel, Duren, Germany). Binary solvent systems acetonitrile-water was used as the mobile phase:

a) The flow rate of eluent acetonitrile-water (40:60 *v/v*) was 1mL min^{-1} at different temperatures of 30, 35, 40 and 45°C.

b) The flow rates of eluent acetonitrile-water (60:40 *v/v*) were: 0.3, 0.6, 0.8. and 1mL min^{-1} at the temperature of 40°C.

The retention factor, *k*, was calculated as follows:

$$k = \frac{t_r - t_0}{t_0} \qquad (1)$$

where t_r is the retention time of the solute and t_0 is the column void time. Each t_r value was measured three times and then the average value was calculated. The correlation analysis was conducted and all calculations were made using the computer program Origin 6.1.

RESULTS AND DISCUSSION

The following two types of compounds were studied: 17-halogen-16, 17-secoestra-1, 3, 5 (10)- triene derivatives hydroxylated at the 3-position (odd numbered compounds) and their 3-benzyloxy counterparts (even numbered compounds), Figure 1.

Retention Behaviour of 17-halogen-16,17-secoestra-1, 3, 5 (10)-triene derivatives in C-18 Columns with Eluent Acetonitrile-Water.

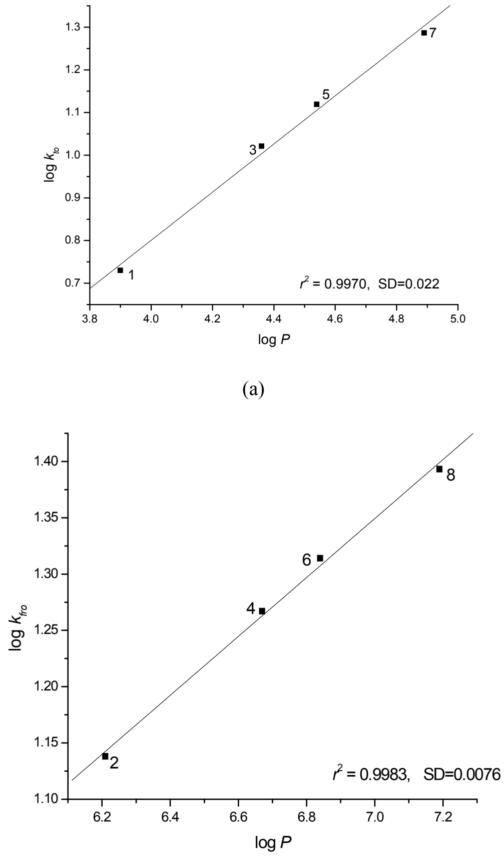
As expected, the odd numbered compounds were generally less retained compared to the even numbered compounds since the benzyloxy group is more hydrophobic in comparison to the hydroxy group. The retention order of 17-halogen-16,17-secoestra-1,3,5(10)-triene derivatives is in accordance with their hydrophobicity. In both mixtures the peaks always emerged in the following order: fluoro-, chloro-, bromo- and iodo- derivatives. This is in accordance with the earlier research we made (Ačanski et al., 2010).

The relationship between the logarithmic retention constant, log *k*, of the compounds in mixture I (compounds 1, 3, 5 and 7) and column temperature (30, 35, 40 and 45°C) for the mobile phase ACN-H_2O (40:60, v/v) was linear. Correlation coefficients of the linear regression analysis of the experimental log *k* values varied from 0.9956 to 0.9971.

Table 1- Retention constants, log k_{to}, log k_{fro}, slopes and correlation coefficients of the linear relationships. Compound numbering is the same as in Figure 1.

a) Temperature (°C)	30	35	40	45	Intercept log k_{to}	Slope	r^2 ± SD
Compound	log k_t						
1	0.467	0.415	0.370	0.334	0.730	-0.009	0.9967 ± 0,006
3	0.723	0.680	0.617	0.579	1.021	-0.009	0.9971 ± 0,008
5	0.810	0.763	0.698	0.660	1.119	-0.010	0.9962 ± 0,008
7	0.956	0.910	0.840	0.797	1.286	-0.011	0.9956 ± 0,008
b) Flow rate (ml min^{-1})	0.3	0.6	0.8	1	Intercept log k_{fro}	Slope	r^2 ± SD
Compound	log k_{fr}						
2	*1.084*	0.748	0.604	0.485	1.138	-0.658	0.9985 ± 0.010
4	*1.228*	0.903	0.767	0.657	1.267	-0.615	0.9981 ± 0.106
6	*1.277*	0.956	0.821	0.714	1.314	-0.605	0.9978 ± 0.011
8	*1.277*	0.956	0.821	0.714	1.393	-0.585	0.9978 ± 0.010

Numerical data for the values of intercepts (log k_{to}) and slopes for each compound studied are presented in Table 1. The relationship between the logarithmic retention constant, log *k*, of the compounds in mixture II (compounds 2, 4, 6 and 8) and flow rates (0.3, 0.6, 0.8 and 1 ml min^{-1}) of the mobile phase ACN-H_2O (60:40, v/v) was parabolic. The values of log *k* for the flow rate of 0.3 ml min^{-1} can be ignored (Italic number in Table 1), and then a parabolic relationship becomes linear. Correlation coefficients of the linear regression analysis of the experimental log *k* values varied from 0.9978 to 0.9985. Numerical data for the values of intercepts (log k_{fro}) and slopes for each compound studied are presented in Table 1.



(a)

(b)

Fig. 2. a) Plot of log k_{to} against log *P* on C-18 column for the mobile phase acetonitrile-water (40:60 *v/v*). b) Plot of log k_{fro} against log *P* on C-18 column for the mobile phase acetonitrile-water (60:40 *v/v*). Compound numbering is the same as in Figure 1.

Correlation between Retention Constant log k_{to} and log k_{fro} of 17-halogen-16,17-secoestra-1,3,5(10)-triene Obtained on C-18 Column and *ACD/*log *P*

Correlations on C-18 column between the retention constant log k_{to} of the 17-halogen-16,17-secoestra-1,3,5(10)-triene and log k_{fro}, with log *P* calculated by the *ACD/*log*P* method are presented in Figures 2a and 2b. Correlation coefficients of the linear regression for Figure 2a and Figure 2b are 0.9970 ± 0.022 and 0.9983 ± 0.007, respectively.

Conclusion

The paper confirmed that there was an excellent correlation between the extrapolated retention constants log k_{to} and log k_{fro} of steroid compounds determined by RPLC on C-18 columns with eluent acetonitrile-water and calculated log *P* using the ACD/log *P* method.

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