RETENTION AND LIPOPHILICITY OF NEWLY SYNTHESIZED 3-BENZYLOXY STEROID DERIVATIVES IN NORMAL- AND REVERSED-PHASE HPLC

Marijana M. Ačanski[1], Đura N. Vujuć[2], Zvonko B. Nježić[2], Suzana S. Jovanović-Šanta[3]

[1]Department of Applied and Engineering Chemistry, Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia

[2]FINS, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia

[3]Department of Chemistry, Biochemistry and Environmental protection, Faculty of Science, University of Novi Sad Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

ABSTRACT

The retention behaviour and separation ability of nine newly synthesized 3-benzyloxy estrone derivatives were studied by HPLC on silica and C-18 commercially available columns. The mobile phases used were: benzene-ethyl acetate, benzene-tetrahydrofuran, benzene-acetonitrile, methanol-water and acetonitrile-water in various proportions. The results are discussed in terms of nature of the solute, eluent and stationary phase.

The correlation between the retention constants of nine 3-benzyloxy estrone derivatives obtained on C-18 column and calculated log *P* was examined too.

1. INTRODUCTION

Steroids are of great interest owing to their varied biological activity. The type of biological activity can changed by introduction and/or changing hydrophilic and hydrophobic functional groups, as well as by transforming the skeleton of the steroid molecule. Steroids are of interest in chromatographic investigations as they offer the opportunity to study the effect of substituents on retention [1-3].

As a continuation of our work on steroid molecule nine new estrogen derivatives have been synthesized in order to functionalize ring A and D of the skeleton, and hence attempt to change the hormonal activity of estrone. Estrogen hormones are female hormones and they promote primary, as well as secondary female characteristics. They play important role in the function of brain, bones, liver, and cardiovascular system [4, 5].

On the other hand development of steroidal compounds is often followed by research on structure-activity relationship studies. For initial chemical screening of activity of newly synthesized compounds, it is first recommended to determine their lipophilicity. Lipophilicity is quantitatively characterized as log *P* (the logarithm of the ratio of the concentrations of any analyte in a saturated 1-octanol-water system) [6-9]. Many methods for estimating log *P*, experimental as well as computational, are described in the literature [10]. The traditional experimental method for the detrmination of log $P_{o/w}$ is *shake flask* method [11]. Nowadays liquid chromatography has a tendency to replace tedious and poor interlaboratory reproducible *shake flask* method for measuring partition coefficients. Among liquid chromatography methods reversed-phase liquid chromatography (RPLC) is an alternative technique that can correlate the hydrophobicity of compounds with the retention parameters [12-14].

In addition to the experimental method, a number of other methods for calculation of 1-octanol-water partition coefficients have been estabilished.

Because of our interest in the biological activity of functionalized newly synthesized steroids and their future derivatives, our study had two objectives:

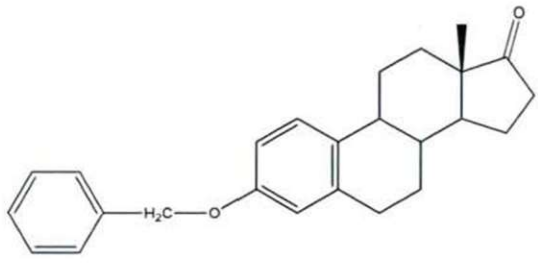
1.reports of the separation performance of normal (silica gel column) and reversed phase (C-18 column) HPLC of newly synthesized steroid compounds, and compares their retention behaviour, and

2. investigation correlation of chromatographically obtained constants in RPHPLC with calculated log *P* values [15].

The structures of the compounds investigated are presented in Table 1.

Table 1. The chemical structure of the compounds studied

1.



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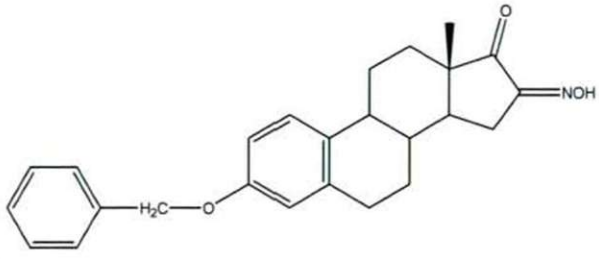

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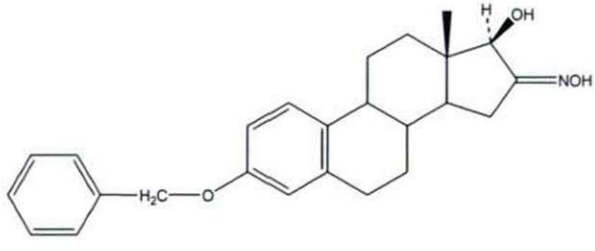

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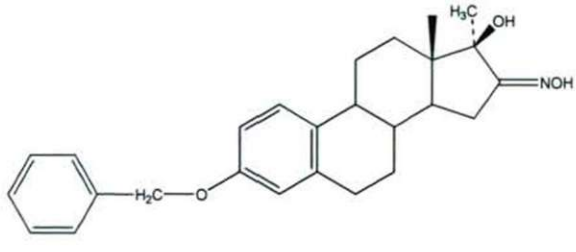

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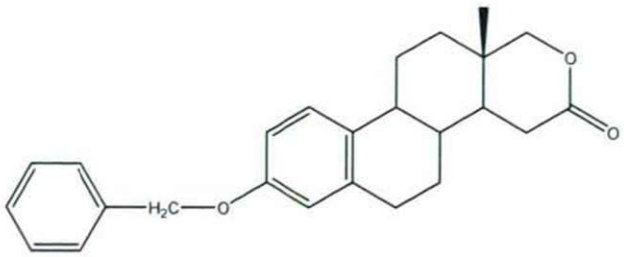
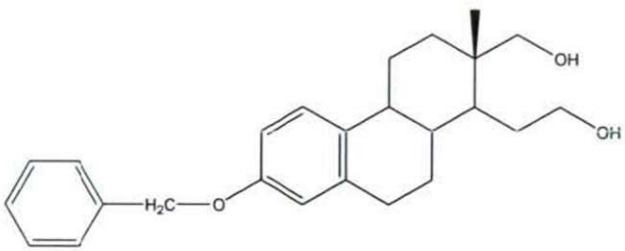

O
O
H2C
O

6.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OH
H2C
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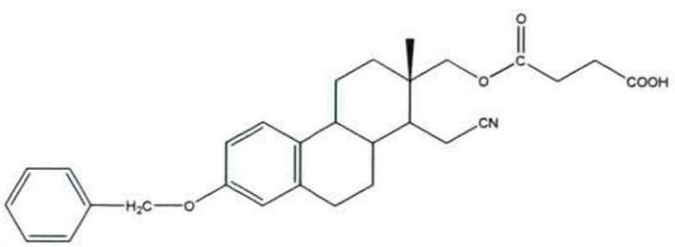
7.


O
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COOH
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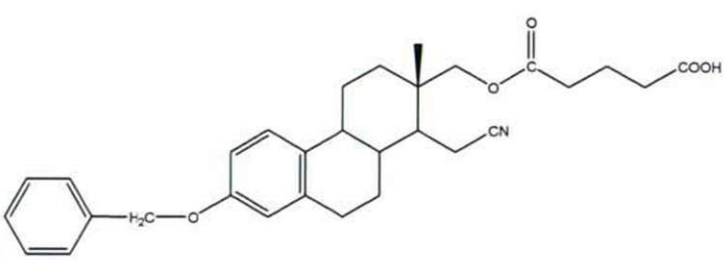
8.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COOH
CN
H2C
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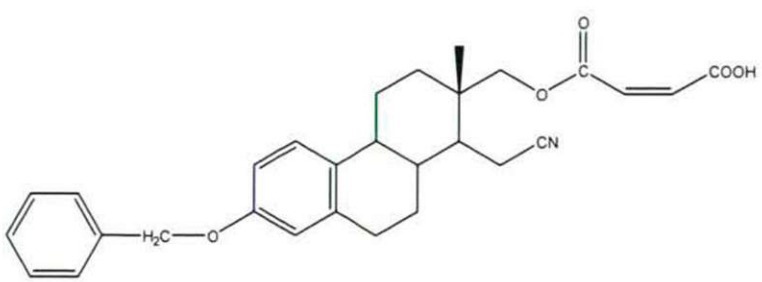
9.


O
O
C
COOH
CN
H2C
O

IUPAC names of steroids:
1. 3-Benzyloxyestra-1,3,5(10)-trien-17-one
2. 3-Benzyloxyestra -1,3,5(10)-triene-16,17-dione 16-oxime
3. 3-Benzyloxy-17β-hydroxyestra-1,3,5(10)-trien-16-one oxime
4. 3-Benzyloxy-17α-methyl-17β-hydroxyestra-1,3,5(10)-trien-16-one oxime
5. 3-Benzyloxy-17-oxa-D-homo-estra-1,3,5(10)-triene-16-on
6. 3-Benzyloxy-16,17-secoestra-1,3,5(10)-triene-16,17-diol
7. 3-Benzyloxy-17-succinoyloxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile
8. 3-Benzyloxy-17-glutaroyloxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile
9. 3-Benzyloxy-17-maleyloxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile

2. EXPERIMENTAL

HPLC separations were performed on an Agilent 1100 Series HPLC (USA) including a degasser G1379 A, binary G1312 pump, ALS G1313A, COLCOM G1316A and DAD G1315B. The columns used were commercially available particle size 5 μm: Spherisorb SI 250 × 4 mm i.d. (E. Merck, Darmstadt, Germany) and Spherisorb ODS-2.5μm, 124 × 4 mm (Hewlett Packard, USA).

Steroid derivatives (Table 1), synthesized by original reactions or according to the literature methods [4, 5], were dissolved (0.05 mg mL^{-1}) in methanol, and the solutions filtered through a 0.2 μm Chromafil filter (Macherey-Nagel, Duren, Germany).

Three binary solvent systems were used as a mobile phase on silica gel column:

(**A**) benzene – ethyl acetate (0.02-0.25, increment, 2 and 5%)

(**B**) benzene – tetrahydrofuran (0.02-0.25, increment 2 and 5%)

(**C**) benzene – acetonitrile (0.02-0.25, increment 2 and 5%)

Two binary solvent systems were used as a mobile phase on octadecyl silica gel column:

(**D**) methanol – water (0.80-0.95, increment 5%)

(**E**) acetonitrile – water (0.70-0.90, increment 5%)

The eluents used to prepare mobile phases were of analytical grade. The flow rate was 1mL min^{-1} at room temperature.

The retention factor, k, was calculated from $k = \frac{t_r - t_0}{t_0}$, where t_r is the retention time of the solute and t_0 the column void time of methanol. Each t_r value was measured in triplicate and averaged.

The correlation analysis was performed by the use of the computer program Origin 6.1.

3. RESULTS AND DISCUSSION

All 3-benzyloxy estrone derivatives were examined by normal- and reversed-phase HPLC on silica gel and C-18 bonded silica gel columns. Detailed discussion of all experiments performed is not necessary and shall concentrate on the most important observations.

3.1. Normal- phase chromatography

The change of retention of the steroid compounds with the change of the volume rate of the more polar component in the mobile phase is in accordance with the well known equation Eq. (1), Fig.1.:

$$\log k = \log k_0 - n \log \varphi \qquad (1)$$

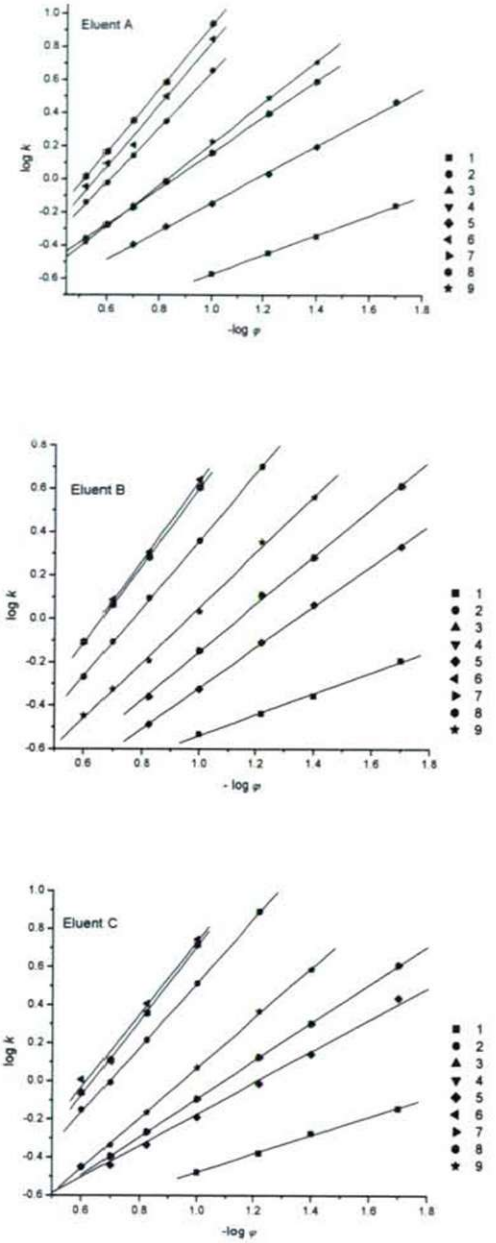


Figure 1. Correlation lines of equation (1), for eluents A-C. Designation of solutes is as in Table 1.

The coefficients log k_0 and n are presented in Table 2.

Table 2. Constants n and log k_0 of the linear relation between retention and eluents A-C composition. Designation of solutes is as in Table 1.

Compound	Eluent A		Eluent B		Eluent C	
	log k_0	n	log k_0	n	log k_0	n
1	-1.1650	0.590	-1.0256	0.486	-0.9632	0.484
2	-1.0253	1.674	-1.2036	1.563	-1.1858	1.699
3	-0.9965	1.930	-1.1918	1.795	-1.2577	1.967
4	-0.9965	1.930	-1.1918	1.795	-1.2577	1.967
5	-0.9998	0.586	-1.2621	0.941	-09999	0.824
6	-1.0562	1.885	-1.2070	1844	-1.1986	1.925
7	-0.9243	1.082	-1.2565	1.103	-1.0946	1.000
8	-0.9243	1.082	-1.2565	1.103	-1.0946	1.000
9	-1.0331	1.246	-1.2289	1.280	-1.2478	1.315

The correlation coefficients from linear regression analysis of experimental log k values varied from 0.9931 to 0.9999. On silica gel the retention sequence of 3-benzyloxy estrone derivatives obtained with non-polar eluents is that predicted on the basis of polarity of the compounds. The sequence of separation on the silica gel column with eluents A-C of all compounds is:

$$\mathbf{3 = 4 \geq 6 > 9 > 7 = 8 > 2 > 5 > 1}$$

The most retained compounds were compounds **3**, **4** and **6**. They are poorly or not resolved. Compounds **3** and **4** have in positions 16 and 17 polar =NOH and –OH groups. In same positions compound **9** has two –OH groups. Non polar 17α-methyl group of compound **4** did not affect retention on silica gel. The least retained compound was compound **1**. Compounds **7** and **8** were not resolved because the number of non-polar methylene groups in alkyl chain in position 17 did not affect the retention, and they always move together. Compound **9** always had a stronger retention then compounds **7** and **8**. With eluent B, the resolution of the midlle polarity compounds (**9**, **7**, **8**, **2**, **5**) was the best; the straight lines of all compounds did not intersect each other (Fig. 1).

The constant n in the equation (1) depends directly on polarity compounds. The more polar derivatives have higher values of the constant n and vice versa. The constant log k_0 is the value log k extrapolated to φ =1 and, therefore, no correlation was found between the constants n and log k_0.

3.2. Reversed- phase chromatography

The change in compounds retention of the steroid derivatives with increasing volume fraction of the modifier in aqueous mobile phases was in accordance with the well known equation, generally accepted in partition chromatography, Eq. (2):

$$\log k = \log k_0 - s\varphi \qquad (2)$$

where φ is the volume fraction of the organic component of the binary aqueous mobile phase, log k_0, is the value of log k extrapolated to φ = 0, and s is constant. The relationship between retention factor, log k, of the investigated compounds and volume fraction, φ, of the modifier in the aqueous mixture was linear, Fig. 2.

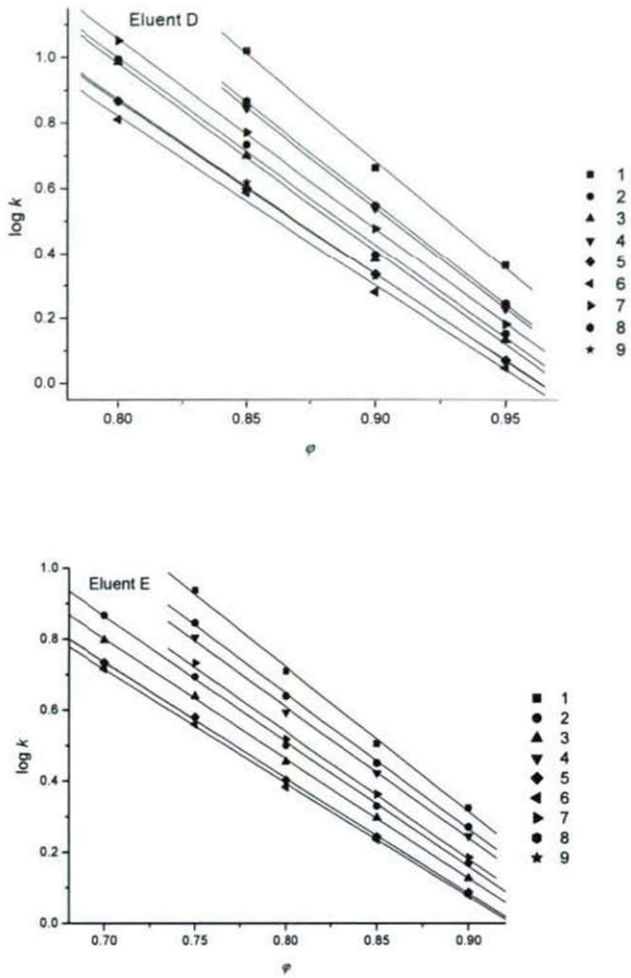


Figure 2.Correlation lines of equation (2), for eluents D and E. Designation of solutes is as in Table 1.

The compounds were more mobile in eluent with acetonitrile as the eluent modifier than with methanol owing to the lower polarity of acetonitrile. It is empirically known that a change from methanol to acetonitrile generally decreases the selectivity.

The numerical values of the absolute value constants s and log k_0 for each compound examined and mobile phases containing water and methanol or acetonitrile as modifier are given in Table 3. Correlation coefficients from linear regression analysis of experimental log k values varied from 0.9979 to 0.9999.

Table 3. Constants s and log k_0 of Equation (2) for the linear relationship between retention and mobile phases composition. Designation of solutes is as in Table 1.

Compound	Eluent D		Eluent E		Log *P*
	log k_0	-*s*	log k_0	-*s*	
1	6.6043	6.580	3.9907	4.086	5.375
2	5.5865	5.574	3.3324	3.526	4.724
3	5.5835	5.572	3.1624	3.374	4.909
4	6.0913	6.170	3.5709	3.702	5.356
5	5.1110	5.306	3.0204	3.264	5.489
6	4.9815	5.200	2.9530	3.198	4.809
7	5.7095	5.816	3.4181	3.598	6.022
8	6.1510	6.220	3.7101	3.828	6.527
9	5.1680	5.368	3.0120	3.254	6.309

The retention data obtained on C-18 bonded silica gel column are generally typical of reversed phase chromatographic behaviour: less polar solutes are more strongly retained. The retention order of the compounds with both aqueous mobile phases was very similar and increases in the following order:

1 > 8 > 4 > 7 > 2 ≥ 3 > 9 ≥ 5 > 6

Compounds **3, 4** and **6** as well as **7** and **8** which were not resolved on silica gel column were clearly resolved on C-18 bonded silica gel (Fig. 2), because retention of compounds is determined with hydrophobicity of compounds. Compounds **2** and **3** were poorly resolved because in reversed-phase the retention is determined by the benzyloxy function only, *i. e.*, the polar keto and hydroxy groups at position 17 did not affect the retention [3].

It is apparent from the data in Table 3 that on C-18 bonded silica gel column with both mobile phases the constant log k_0 and the absolute value of the constant *s* increase with increasing compound retention. There are, therefore, linear relationships between these two constants, with high correlation coefficients, Fig. 3.

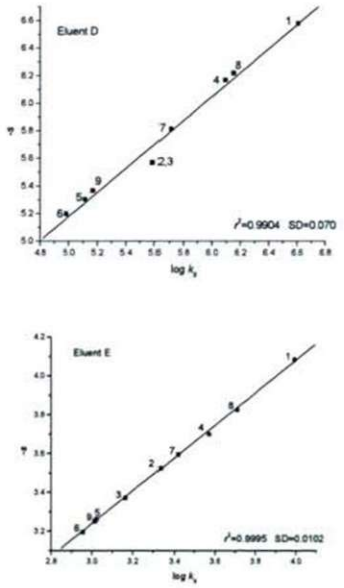


Figure 3. Plot of log k_0 against s for the mobile phases D and E. Designation of compounds is as in Table 1.

3.3. Correlation Between Retention Constant log k_0 of Steroid Derivatives Obtained on 7C-18 Column and log P

The intercept log k_0 corresponds to the retention in water as mobile phase, and represents the commonly employed chromatographic hydrophobicity parameter [10, 14].

With respect to the nine newly synthesized steroid compounds does not belong to the same series, therefore, no correlation was found between the constants log P (values log P are given in the last column of Table 3) and log k_0.

4. CONCLUSION

On silica gel the retention sequence of 3-benzyloxy estrone derivatives obtained with non-polar eluents is that predicted on the basis of polarity of the compounds. The change in steroid compounds retention with an increment of volume fraction of the stronger solvent in mobile phase is in accordance with the well know equation generally accepted in adsorbtion chromatography.

On octadecyl silica gel the retention sequence of the compounds obtained by applying aqueous-organic mobile phases, is basically the consequence of hydrophobicity of compounds. The change in compounds retention with an increment of volume fraction of the stronger solvent in mobile phase is in accordance with the well know equation generally accepted in partition chromatography.

For resolving of 3-benzyloxy estrone derivatives it is necessary to have one column of octadecyl silica gel and one column of silica gel. This combination guarantees resolution and makes possible quantitative determination of the steroid derivatives.

No correlation was found between the constants log P (values log P are given in the last column of Table 3) and log k_0, because nine newly synthesized steroid compounds does not belong to the same series.

ACKNOWLEDGEMENT

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