

## Normal and reversed phase thin-layer chromatography of new 16,17-secoestrone derivatives

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*Abstract:* The retention behaviour and separation ability of a series of new 16,17-secoestrone derivatives has been studied on silica gel, alumina and C-18 silica gel layers with non-aqueous and aqueous-organic mobile phases. The retention behaviour and separation ability are discussed in terms of the nature of the solute, eluent and stationary phase.

*Keywords:* thin-layer chromatography, silica gel, alumina, C-18 silica gel, 16,17-secoestrone derivatives.

### INTRODUCTION

In our previous papers,<sup>1–4</sup> the retention behaviour of some estradiol and estrone derivatives chromatographed on: silica gel, chemically bonded phases and C-18 bonded silica gel in normal and reversed phase, using several non-aqueous and aqueous eluents has been described. The type of the stationary and mobile phases, as well as the nature, number, and position of substituents in the molecule of the steroid compounds were observed to have significant and distinct effects on the retention.

As a continuation of this work on steroid molecule transformations, a new series of estrone derivatives has been synthesized in order to functionalize the A and D rings of the estrone skeleton and hence to attempt to change the hormonal activity of estrone.<sup>5</sup>

Therefore, in this work new 16,17-secoestrone derivatives<sup>5</sup> were chromatographed on normal: silica gel and alumina TLC plates and reversed phase C-18 silica gel HPTLC plates.

The compounds and their structures are listed in Table I.

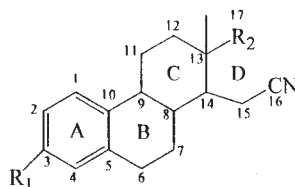
### EXPERIMENTAL

TLC was performed on 20 × 20 cm glass plates precoated with silica gel (Merck), alumina (Alox-25; Macherey-Nagel) and 10 × 10 cm glass HPTLC plates coated with C-18 bonded silica gel (Merck), all with fluorescence indicator.

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TABLE I. Chemical structures of the compounds studied



Compound	R <sub>1</sub>	R <sub>2</sub>
1	OH <sup>a)</sup>	CH <sub>2</sub> OH
2	OCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub> <sup>b)</sup>	CH <sub>2</sub> OH
3	OH	CHO
4	OCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	CHO
5	OH	CH <sub>2</sub> OSO <sub>2</sub> C <sub>6</sub> H <sub>4</sub> CH <sub>3</sub>
6	OCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	CH <sub>2</sub> OSO <sub>2</sub> C <sub>6</sub> H <sub>4</sub> CH <sub>3</sub>
7	OH	CH <sub>2</sub> F
8	OCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	CH <sub>2</sub> F
9	OH	CH <sub>2</sub> Cl
10	OCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	CH <sub>2</sub> Cl
11	OH	CH <sub>2</sub> Br
12	OCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	CH <sub>2</sub> Br
13	OH	CH <sub>2</sub> I
14	OCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	CH <sub>2</sub> I

<sup>a)</sup> -OH, hydroxy, <sup>b)</sup> -OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, benzyloxy, -OBn

IUPAC names of the steroids:

1. 3, 17 - Dihydroxy -16,17 - secoestra -1, 3,5 (10) triene - 16 - nitrile
2. 3 - Benzyloxy - 17 - hydroxy - 16, 17 - secoestra - 1, 3, 5 (10) triene - 16 - nitrile
3. 3 - Hydroxy - 17 - oxo - 16, 17 - secoestra - 1, 3, 5 (10) triene - 16 - nitrile
4. 3 - Benzyloxy - 17 - oxo - 16, 17 - secoestra - 1, 3, 5 (10) triene - 16 - nitrile
5. 3 - Hydroxy 17 p - toluenesulfonyloxy 16,17 - secoestra - 1, 3, 5 (10) triene - 16 - nitrile
6. 3 - Benzyloxy 17p - toluenesulfonyloxy 16, 17 - secoestra - 1, 3, 5 (10) triene - 16 - nitrile
7. 3 - Hydroxy - 17 - fluoro - 16, 17 - secoestra - 1,3,5 (10) triene - 16 - nitrile
8. 3 - Benzyloxy - 17 - fluoro - 16, 17 - secoestra - 1, 3, 5 (10) triene - 16 - nitrile
9. 3 - Hydroxy - 17 - chloro - 16, 17 - secoestra - 1,3,5 - (10) triene - 16 - nitrile
10. 3 - Benzyloxy - 17 - chloro - 16, 17 - secoestra - 1, 3, 5 (10) triene - 16 - nitrile
11. 3 - Hydroxy - 17 - bromo - 16, 17 - secoestra - 1, 3, 5 (10) triene - 16 - nitrile
12. 3 - Benzyloxy - 17 - bromo - 16, 17 - secoestra - 1,3,5 (10) triene - 16 - nitrile
13. 3-Hydroxy - 17 - iodo - 16, 17 - secoestra - 1, 3, 5 (10) triene - 16 - nitrile
14. 3 - Benzyloxy - 17 - iodo - 16, 17 - secoestra - 1, 3, 5 (10) - triene - 16 - nitrile

The compounds were dissolved in methanol (2 mg mL<sup>-1</sup>) and 1 μL volumes of the solutions were spotted randomly on the layers.

The mobile phases used are listed in Table II. At least three chromatograms were developed for each solute-solvent combination and the R<sub>F</sub> values averaged. The R<sub>M</sub> values were calculated from:

$$R_M = \log \left( \frac{1}{R_f} - 1 \right)$$

The spots were observed under UV light at  $\lambda = 254$  nm.

#### RESULTS AND DISCUSSION

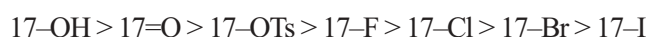
Two types of compounds were studied: 3-hydroxy (3-OH) derivatives of 16,17-secoestrone derivatives (compound with odd numbers) and 3-benzyloxy (3-OBn) of 16,17-secoestrone derivatives (compounds with even numbers) (Table I); all were systematically examined by normal and reversed phase TLC on silica gel, alumina and C-18 HPTLC modified silica gel. The retention data are collected in Table II.

TABLE II. The solvent systems (v/v) used as the mobile phase

	Mobile phases	Silica gel	Alumina
I <sub>1</sub>	Hexane-ethyl acetate	8:2	7:3
I <sub>2</sub>	Hexane-acetone	8:2	7:3
II <sub>1</sub>	Cyclohexane-ethyl acetate	7.5:2.5	7.5:2.5
II <sub>2</sub>	Cyclohexane-acetone	7.5:2.5	7.5:2.5
III <sub>1</sub>	Benzene-ethyl acetate	9.5:0.5	9.5:0.5
III <sub>2</sub>	Benzene-acetone	9.5:0.5	9.5:0.5
	Mobile phases	Bonded C-18 silica gel	
IV <sub>1</sub>	Methanol-water	8:2	
IV <sub>2</sub>	Acetonitrile-water	8:2	

#### Normal phase chromatography

*Silica gel.* On silica gel layers with eluents I<sub>1</sub>–III<sub>2</sub> (Table II), the compounds with odd numbers were generally more retained in comparison with the compounds with even numbers, because the hydroxy group is much more polar than the benzyloxy group (Table III). The most retained compound was, as expected, compound **1**. The retention of compounds possessing a halogen atom at position 17 was low and decreased from fluorine to iodine. The unusual substituent toluensulfonyloxy group (compounds **5** and **6**) had an affect which was between the affect of polar groups and the affect of halogen atoms. Therefore, the retention order of the new 16,17-secoestrone derivatives in all the investigated systems was more or less the same, for both types of compounds, and increased in the order:



As all the studied compounds possessed an aromatic A ring, the enhanced solubility of the solutes in benzene resulted in lower retention when the hexane and cyclohexane in the eluent was substituted with benzene. The type of diluent (hexane, cyclohexane and benzene) did not affect the general retention behaviour of the investigated compounds. This

is not the case with eluents (ethyl acetate and acetone). Although acetone is more polar than ethyl acetate, some of compounds were more retained in the mobile phase with acetone than with ethyl acetate. Thus, certain retention data (Table III) show that the retention on silica gel does not always indicate the real polarity of the compound.

The differences between the retention constants ( $\Delta R_M$ ) when the 3-OH group was substituted the 3-OBn group function for various solute pairs (solute pairs 1-2, 3-4, 5-6, 7-8, 9-10, 11-12, 13-14) are more or less constant in some cases for the particular mobile phases, but in some cases are very different. Therefore, it was not possible to establish a mutual dependence between the value of  $\Delta R_M$  of the compared pairs and the mobile phases.

*Alumina.* The  $R_M$  values calculated for the new 16,17-secoestrone derivatives are presented in Table III. The more polar derivatives, compounds **1** and **3**, were strongly retained or stayed at start of the chromatogram. Less polar derivatives, compounds **8**, **10**, **12**, **14**, had very low retention. With the mobile phases III<sub>1</sub> and III<sub>2</sub>, compounds **8**, **10**, **12**, **14**, moved with the front. The general retention behaviour of the new 16,17-secoestrone derivatives on alumina was similar to the retention behaviour on silica gel. Hence a detailed discussion is not necessary. The  $\Delta R_M$  values of the solute pairs on alumina were much higher than on silica gel. For this reason it was impossible to resolve all the compounds with same mobile phase, on the plate with eluents III<sub>1</sub> and III<sub>2</sub>.

#### *Reversed phase (RP) chromatography*

*Bonded C-18 silica gel.* On the basis of the  $R_M$  values (Table III), in eluents IV<sub>1</sub> and IV<sub>2</sub> it was shown that the retention behaviour of the new 16,17-secoestrone derivatives is in accordance with their polarity. As expected, the compounds with odd numbers were generally less retained in comparison with the compounds with even numbers; because the phenyl ring is more hydrophobic compared to the hydroxy group. Namely, less polar solutes were more strongly retained and *vice versa*.

Although methanol and acetonitrile showed similar behaviour with regard to the retention order of the compounds, one exception was observed. Compounds **5** and **6**, possessing the toluensulfonyloxy function at position 17 were significantly more retained than compounds **7** and **8** bearing fluorine. This rule observation was pronounced in the retention behaviour of compounds **5** and **7** when the mobile phase was acetonitrile–water too, but was not pronounced for compounds **6** and **8**. Namely, compound **6** was less retained with the acetonitrile–water mobile phase than compound **8**.

Therefore, the retention order of the new 16,17-secoestrone derivatives in both the investigated systems in reversed phases was very similar for both types of compounds, and increases in the order:



The compounds were more mobile in eluent IV<sub>2</sub> than in eluent IV<sub>1</sub> owing to the lower polarity of acetonitrile. It is known empirically that a change from methanol to acetonitrile generally decreases the selectivity.<sup>6,7</sup> Therefore, the values of  $\Delta R_M$  for the

TABLE III.  $R_M$  values of compounds **1** – **14** (Table I) on silica gel and alumina with mobile phase I<sub>1</sub>–III<sub>2</sub> and on C-18 bonded silica gel with mobile phases IV<sub>1</sub> and IV<sub>2</sub>. Values of  $\log P$  of compounds **1** – **14** are presented in last row of this Table III.

Mobile phase	$R_M$ of compound													
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>
Chromatography on silica gel														
I <sub>1</sub>	1.440	1.158	0.886	0.399	1.032	0.513	0.513	0	0.501	-0.035	0.537	-0.043	0.513	-0.035
I <sub>2</sub>	1.123	0.644	0.865	0.432	0.704	0.149	0.673	0.167	0.673	0.149	0.673	0.176	0.659	0.176
II <sub>1</sub>	0.931	0.616	0.421	-0.061	0.465	-0.017	0.149	-0.368	0.122	-0.421	0.113	-0.443	0.087	-0.501
II <sub>2</sub>	0.807	0.489	0.525	0.087	0.673	0.231	0.432	-0.149	0.389	-0.167	0.410	-0.213	0.399	-0.240
III <sub>1</sub>	1.380	0.931	0.562	-0.122	0.537	-0.213	0.278	-0.753	0.269	-0.770	0.259	-0.788	0.250	-0.845
III <sub>2</sub>	1.032	0.602	0.288	-0.501	0.269	-0.525	0.017	-1.235	0.026	-1.380	0.035	-1.510	0.052	-1.558
Chromatography on alumina														
I <sub>1</sub>	1.69	0.807	1.005	-0.131	1.123	-0.105	0.770	-0.589	0.788	-0.575	0.753	-0.575	0.704	-0.753
I <sub>2</sub>	0.753	0.454	0.720	0.105	0.865	0.158	0.602	0.122	0.550	-0.194	0.573	-0.176	0.575	-0.194
II <sub>1</sub>	1.591	0.826	1.032	-0.096	1.091	-0.113	0.720	-0.589	0.689	-0.630	0.659	0.788	-0.644	-0.865
II <sub>2</sub>	1.158	0.525	0.807	0.105	0.865	0.185	0.659	-0.176	0.644	-0.259	0.616	-0.288	0.602	-0.347
III <sub>1</sub>	1.996	0.845	1.061	-0.602	1.235	-0.704	0.954	-	0.908	-	0.886	-	0.865	-
III <sub>2</sub>	1.690	0.865	0.954	-0.865	0.954	-1.158	0.575	-	0.673	-	0.704	-	0.689	-
Chromatography on C-18 silica gel														
IV <sub>1</sub>	-0.213	0.525	-0.176	0.630	0.140	0.954	0.061	0.826	0.176	1.005	0.231	1.061	0.269	1.091
IV <sub>2</sub>	-0.788	-0.203	-0.770	-0.167	-0.525	0.026	-0.602	0.087	-0.501	0.140	-0.454	0.158	-0.410	0.194
$\log P$	2.72	5.03	2.99	5.30	4.08	6.39	3.90	6.21	4.36	6.67	4.54	6.84	4.89	7.19

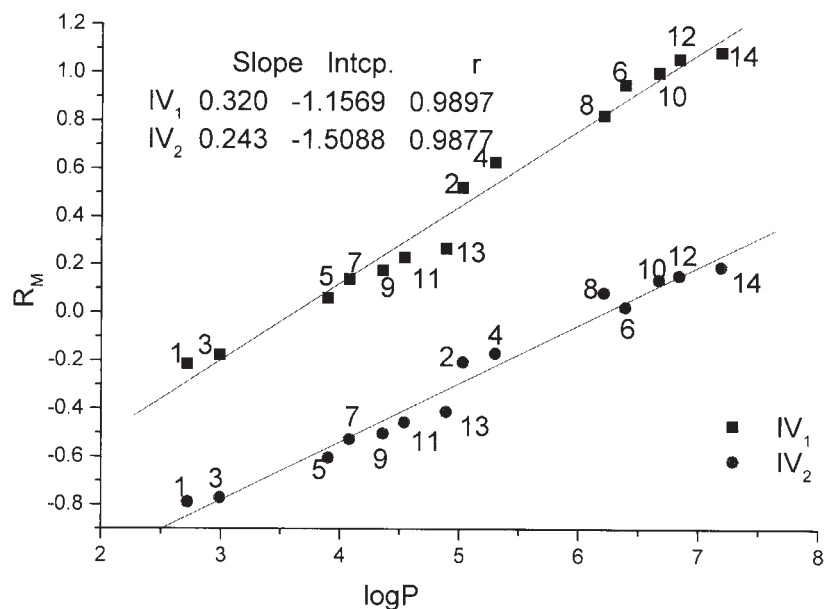


Fig. 1. Linear relationship between  $\log P$  and the retention constant  $R_M$  in mobile phases IV<sub>1</sub> and IV<sub>2</sub>. Compound numbering as in Table I.

compound pairs with substitution of the 3-OH with the 3-OBn function are higher in eluent IV<sub>1</sub> than in eluent IV<sub>2</sub>. The average  $\Delta R_M$  value for eluent IV<sub>1</sub> is  $0.801 \pm 0.034$ , while the average  $\Delta R_M$  value for eluent IV<sub>2</sub> is  $0.612 \pm 0.047$ .

In recent years, partition chromatography, especially RPTLC, has become a popular alternative for measuring lipophilicity. Some authors successfully performed extrapolations of  $R_M$  data to 100 % water conditions ( $\Delta R_{MW}$ ).<sup>8,9</sup> Exactly the opposite appeared in the investigations of some other authors, since correlations between  $\log P$  and chromatographic indices were better in the case of non-extrapolated data (50 to 60 modifier conc.) as compared to  $R_{MW}$  data.<sup>10-13</sup>

As the retention of a compound in reversed-phase chromatography is governed by hydrophobic interactions, linear relationships between the retention constant  $R_M$  and  $\log P$  (calculated by computer program ACD  $\log P$ , Table III) could be expected.

The obtained relationships are presented in Fig. 1.

## ИЗВОД

ТАНКΟΣЛОЈНА ХРОМАТОГРАФИЈА НА НОРМАЛНИМ И ОБРНУТИМ ФАЗАМА  
НЕКИХ НОВИХ ДЕРИВАТА 16,17-СЕКОЕСТРОНАМАРИЈАНА М. АЧАНСКИ<sup>1</sup>, СУЗАНА ЈОВАНОВИЋ-ШАНТА<sup>2</sup> и ЛИДИЈА Р. ЈЕВРИЋ<sup>1</sup>

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У раду је испитано ретенционо понашање и раздвајање серије новосинтетизованих 16,17-секоестронских деривата на: силика гелу, алуминијум-оксиду и модификованом октадецил силика гелу, применом неводених и водених покретних фаза. Ретенционо понашање и раздвајање испитиваних деривата је дискутовано са аспекта природе растворка, непокретне и покретне фазе.

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