J.Serb.Chem.Soc. 68 (3)163–170(2003)
JSCS – 3031

UDC 549.73+577.175.6+661.183.7:543.544
Original scientific paper

Retention behaviour of some estradiol derivatives on alumina in normal phase chromatography

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(Received 24 September, revised 8 November 2002)

Abstract: The retention constants of variously substituted estradiol derivatives were measured as a function of the composition of various binary mobile phases in order to study the relationship between chemical structure and retention behaviour in TLC on alumina. The slopes and intercepts of the linear relationships between the retention constant R_M and the logarithm of the volume fraction of the polar mobile phase component were calculated and are discussed in relation to the characteristics of the solute and mobile phase. The R_F values and relative retention ΔR_M of the compounds depend largely on the retention behaviour of their substituents. The results obtained in this investigation are compared with the results of the same derivatives obtained in previous investigations on silica gel.

Keywords: thin layer chromatography, alumina, non-aqueous binary mobile phases, estradiol derivatives, Soczewinski equation.

INTRODUCTION

Estradiol is the most important human estrogen produced by the ovaries. Other estrogens (estrone, estriol) may be weaker, but in principle have the same effect. The importance of the functional groups on the activity of natural estradiol has been recognized. Some simple chemical modification of the basic structure of estradiol can have a direct effect on their activity, primarily by modification of the binding activity of the steroid to the receptors. Thus, small conformational changes in estrogens play an important role in the design of new estrogens and antiestrogens.[1,2]

One series of estradiol derivatives has been synthesized in order to functionalize the A, B and D rings of estradiol skeleton, in an attempt to change the hormonal activity of estradiol.[3,4]

In our previous papers[5–8] the retention behaviour of the same estradiol derivatives chromatographed on a variety of stationary phases: silica gel, chemically bonded phases in normal and reversed phase, using several non-aqueous and aqueous eluents has been described.

Serbian Chemical Society active member.

The most successful models of retention in liquid-solid chromatography are those of Snyder *et al.*[9] and Soczewinski.[10–13] In actuality, these models are quite similar. Both of them assume that retention is the product of competitive adsorbtion between the solute and mobile phase molecules for the active sites on the stationary-phase surface.

The Soczewinski equation:

$$R_M = R^0_M - n \log \varphi \quad (1)$$

where φ is the volume fraction of a polar component of a binary eluent and R^0_M and n are constants. Constant n is widely observed. Snyder *et al.* predicts that the constant n, *i.e.*, the slope of this line, should be the ratio of the molecular areas of the solute and mobile phase, whereas the Soczewinski's model predicts that this slope is the number of strongly adsorbing substituent groups on the solute. Petrović *et al.*[5,14–16] showed for a series of steroidal compounds and electron donor solvents that the constant n depends on the type and the number of substituents in the molecule, as well as on the skeleton structure; it is the sum of the particular Δn values. They also showed that the slope linearly increases with increasing value of the constant ΔR^0_M and that when an aliphatic hydrocarbon as diluent is substituted with an aromatic hydrocarbon, the slope of Eq. (1) decreases approximately 1.5-fold.

Therefore, in this work Eq. (1) was applied to study the retention behaviour of some synthesized[3,4] estradiol derivatives on alumina layers using non-aqueous binary mobile phases. The retention data of estradiol derivatives collected in this work are compared with those of the same estradiol derivatives collected in previous investigations on silica gel.[7]

The most important characteristics of these two sorbents are[17–20]:

Silica gel is still by far the most commonly used support material. Silica gel plays a direct role in the retention through interactions between solutes and its surface-active sites. It is generally accepted that the surface-active sites on silica are Si-OH groups (silanols). As a result of steric hindrance, the density of silanols groups on chromatographic-grade silica is $\approx 8 \pm 1$ μmol/m^2.

Alumina is one of the most versatile sorbents for preparative chromatography. On alumina there are two surface-active sites: hydroxy groups and oxygen anions. The average density of hydroxy groups is approximately 13 μmol/m^2. It is a weaker sorbent than silica gel and much less used in chromatography of steroids. Alumina and silica gel have similar chromatographic effects, but alumina has a stronger adsorbtion power for molecules with double C=C bonds and a better selectivity in the resolution of aromatic hydrocarbons and their derivatives.

EXPERIMENTAL

TLC was performed on 20 × 20 cm glass plates precoated with alumina (Alox-25; Macherey-Nagel) with a fluorescence indicator (Merck).

The structures of the estradiol derivatives are given in Table I.

The compounds were dissolved in methanol (2 mg mL^{-1}) and 1 μL volumes of the solutions were spotted randomly on the plates.

The following solvent binary mixtures were used as mobile phases:

(A) benzene – acetone (90 + 10 v/v)
(B) heptane – acetone (70 + 30 v/v)
(C) heptane – ethyl acetate (60 + 40 v/v)
(D) hexane – dioxane (70 + 30 v/v)
and mixtures of the following binary eluents:
(E) heptane – ethyl acetate (40 – 55 %, increment 5 %)
(F) benzene – ethyl acetate (25 – 40 %, increment 5 %)
(G) heptane – acetone (35 – 50 %, increment 5 %)
(H) benzene – acetone (10 – 25 %, increment 5 %)

At last three chromatograms were developed for each solute-solvent combination and R_F values averaged. The R_M values were calculated from $R_M = \log (\frac{1}{R_F} - 1)$.

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

RESULTS AND DISCUSSION

The compounds given in Table I, were systematically examined by TLC on alumina using mobile phases A–D. The retention data are collected in Table II.

The retention sequence obtained with non-polar eluents was predicted on the basis of the polarity of the compounds. The retention sequences of the compounds were more or less similar with mobile phases A–D and the results are in accordance with the general retention behaviour in normal phase chromatography. Namely, the more polar derivatives, compounds **1**, **10** and **14**, were strongly retained or remained at the start of chromatogram. Less polar derivatives, compounds **4**, **6**, **8** and **9**, had very low retention and moved with the front in mobile phases A and D. The other derivatives, compounds **2**, **3**, **5**, **7**, **11** and **15**, had a more or less similar retention order in eluents A–D.

The compounds which were not resolved with one mobile phase were resolved with other mobile phases. For example, in eluents C and D, compounds **7** and **15** moved together, but in eluent A they were clearly resolved, with compound **15** having the bigger retention. This is probably because of the greater solubility of the benzoyloxy substituent in benzene compared to propionoxy substituens.

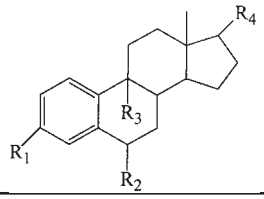
With the eluents A, B and D, compound **4** always had a stronger retention than compounds **8** and **9**. With eluent C, compound **4** had a significantly lower retention, even less than compounds **8** and **9**. This is probably because of the greater solubility of the acetate substituent in ethyl acetate than in the other eluents: acetone and dioxane. This is in accordance with earlier investigations on silica gel.[7]

Thus, the general retention behaviour of the estradiol derivatives on alumina was similar to that on silica gel.[7]

The ΔR_M values of the solute pairs on alumina were much higher than on silica gel (Table III). For this reason it was impossible to resolve all the compounds with all the solvents in the same mobile phases, on the same alumina plates.

The change in retention of the estradiol derivatives with increasing volume fraction of the stronger solvent in the binary mobile phase, *i.e.*, retention mechanism, is in accordance with Eq. (1). These experiments worked only with estradiol derivatives which had R_F

TABLE I. Chemical structures of the compounds studied



Com.	R_1	R_2	R_3	R_4
1.	OH			OH
2.	OCH_3			OH
3.	$OCOCH_3$			OH
4.	$OCOCH_3$			$OCOCH_3$
5.	$OCOCH_2CH_3$			OH
6.	$OCOCH_2CH_3$			$OCOCH_2CH_3$
7.	$OCOC_6H_5$			OH
8.	$OCOC_6H_5$			$OCOC_6H_5$
9.	$OCOCH_3$			$OCOC_6H_5$
10.	OH	=O		OH
11.	OCH_3	=O		OH
12.	OH	=O		$OCOCH_2CH_3$
13.	$OCOCH_2CH_3$	=O		$OCOCH_2CH_3$
14.	OH	=O	α-OH	$OCOCH_2CH_3$
15.	$OCOCH_2CH_3$	=O	α-OH	$OCOCH_2CH_3$

IUPAC names of steroids:

1. 3,17β-Dihydroxyestra-1,3,5(10)-triene (estradiol)
2. 3-Methoxy-17β-hydroxyestra-1,3,5(10)-triene
3. 3-Acetoxy-17β-hydroxyestra-1,3,5(10)-triene
4. 3,17β-Diacetoxyestra-1,3,5(10)-triene
5. 3-Propionoxy-17β-hydroxyestra-1,3,5(10)-triene
6. 3,17β-Dipropionoxyestra-1,3,5(10)-triene
7. 3-Benzoyloxy-17β-hydroxyestra-1,3,5(10)-triene
8. 3,17β-Dibenzoyloxyestra-1,3,5(10)-triene
9. 3-Acetoxy-17β-benzoyloxyestra-1,3,5(10)-triene
10. 3,17β-Dihydroxyestra-1,3,5(10)-triene-6-one (6-oxoestradiol)
11. 3-Methoxy-17β-hydroxyestra-1,3,5(10)-triene-6-one
12. 3-Hydroxy-17β-propionoxyestra-1,3,5(10)-triene-6-one
13. 3,17β-Dipropionoxyestra-1,3,5(10)-triene-6-one
14. 3,9α-Dihydroxy-17β-propionoxyestra-1,3,5(10)-triene-6-one
15. 3,17β-Dipropionoxy-9α-hydroxyestra-1,3,5(10)-triene-6-one

TABLE II. R_F values of steroids on alumina using mobile phases A–D

Compound	Eluents			
	A (0.9+0.1)	B (0.7+0.3)	C (0.6+0.4)	D (0.7+0.3)
	R_F			
1.	0.07	0.03	0.07	0.23
2.	0.34	0.24	0.30	0.68
3.	0.30	0.165	0.22	0.495
4.	0.85	0.455	0.83	0.885
5.	0.37	0.19	0.24	0.575
6.	0.90	0.62	0.76	0.96
7.	0.35	0.165	0.25	0.59
8.	0.935	0.565	0.75	0.92
9.	0.905	0.555	0.71	0.915
10.	0.02	0	0.015	0.065
11.	0.23	0.1	0.16	0.36
12.	0.05	0.06	0.09	0.295
13.	0.82	0.37	0.58	0.78
14.	0	0.015	0	0.08
15.	0.13	0.13	0.25	0.59

TABLE III. ΔR_M values of some estradiol derivative pairs on silica gel and alumina, with some non-aqueous mobile phases, [a)]Results on silica gel were obtained in a previous investigation[7]

Pairs of compounds	ΔR_M			
	Silica gel[a)]	Alumina	Silica gel	Alumina
	Hx:EtAc[b)](0.75+0.25)	Hx:EtAc (0.6+0.4)	Hx:An (0.75+0.25)	Hp:An (0.7+0.3)
1+2	0.375	1.784	0.361	1.009
1+4	0.823	2.841	0.630	1.432
1+10	–1.769	0.335	0.324	–
1+13	0.595	2.292	0.490	1.297
2+4	0.448	1.057	0.630	0.423
3+4	0.246	1.238	0.288	0.626
12+13	0.417	2.182	0.325	2.006
14+15	0.589	–	0.399	2.173

[b)]Hx, hexane; EtAc, ethyl acetate; An, acetone; Hp, heptane

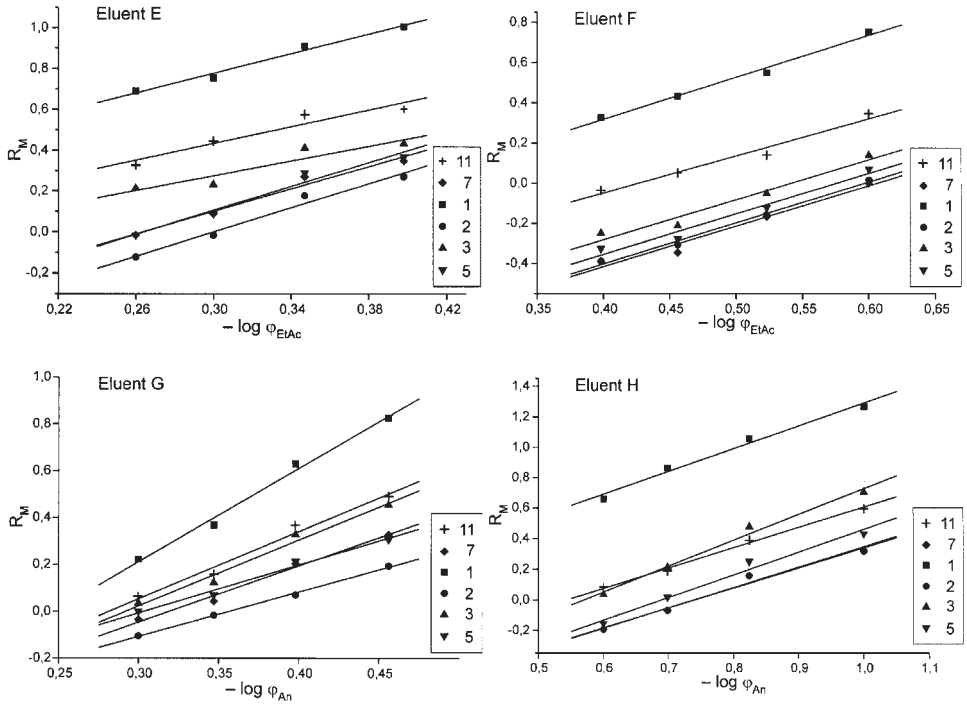


Fig. 1. Correlation lines of equation (1) for eluents E – H. Compound numbering as in Table I.

value in the interval 0.1 to 0.8, *i.e.*, derivatives: **1**, **2**, **3**, **5**, **7** and **11**. The correlation coeficients of the linear regression of the experimental R_M values varied from 0.9361 to 0.9988. The results are presented in Table IV and in Fig. 1.

The type of eluent and the molecular structure of the compounds significantly affected the retention, the slope n of Eq. (1) and, consequently, the selectivity.

In all cases, compound **1** was more clearly resolved than the other compounds in eluents E – H. With eluent F, the resolution of the compounds was the best; the straight lines of all compounds did not intersect each other (Fig. 1).

With eluents E, G and H, the resolution of the compounds was poorer; some straight lines intersected each other. For example, the straight lines of compounds **5** and **7** intersect each other at certain φ values depending on the type of eluent. The intersection point for eluent E is at $\varphi = 0.3$, while for eluent G it is at $\varphi = 0.4$.

Consequently, the selectivity changed with varying φ. In one case, the selectivity was not changed with varying φ. Compounds **2** and **7** always move together with eluent H.

In eluents containing heptane as diluent, the slopes were greater than with those containing benzene. The mean value n for systems with ethyl acetate is 1.24, while for systems with acetone it is 1.85. Therefore, their mean value is approximately 1.5.

The constant n does follow the polarity of the compounds, but not as strictly as was in the case of silica gel.[7]

TABLE IV. Constants n and R^0_M of the linear relation between the retention and the conposition of eluents E–H

Comp.	Eluents							
	E (0.4–0.55)		F (0.25–0.4)		G (0.35–0.5)		H (0.1–0.25)	
	n	R^0_M	n	R^0_M	n	R^0_M	n	R^0_M
1	2.396	0.037	2.087	– 0.516	3.989	– 0.986	1.510	– 0.214
2	2.954	– 0.887	2.044	– 1.212	1.899	– 0.677	1.310	– 0.970
3	1.814	– 0.270	2.002	– 1.083	2.816	– 0.822	1.694	– 0.965
5	2.929	– 0.774	2.023	– 1.164	2.050	– 0.622	1.486	– 1.025
7	2.759	– 0.726	2.019	– 1.224	2.403	– 0.767	1.335	– 0.987
11.	2.049	– 0.182	1.852	– 0.789	2.875	– 0.809	1.331	– 0.723

The constant $R_M{}^0$ is the value R_M extrapolated to $\varphi = 1$ and, therefore, no correlation was found between the constants n and $R_M{}^0$.

CONCLUSIONS

The type of stationary and mobile phases, and nature, number, and position of the substituents in the molecule of the estradiol derivatives were observed to have significant and distinct effects on the retention.

On silica gel[7] the retention sequence of the estradiol derivatives obtained with non-polar eluents is that predicted on the basis of the polarity of the compounds. However, certain retention data showed that the retention on silica gel does not always indicate the real polarity of a compound. The change in retention of the steroid compounds with increasing volume fraction of the stronger solvent in the mobile phase is in accordance with Eq. (1), which is generally accepted in adsorption chromatography. The constant n in Eq. (1) depends directly on the polarity of a compound. The more polar derivatives have higher values of the constant n and *vice versa*. The slope increments, Δn, for the introduction and/or substitution of functional groups in a steroid molecule are reasonably constant for the same substitution and various solute pairs.

On alumina the retention behaviour of the estradiol derivatives is similar to that on silica gel, but the ΔR_M values of solute pairs on alumina are much higher. For this reason, it was impossible to resolve all the estradiol derivatives in the same solute system. The change of the retention for the estradiol derivatives with increasing volume fraction of the polar mobile phase component is in accordance with Eq. (1).

Although the retention behavior of the investigated compounds on alumina is similar to that obtained on silica gel, the constant n is not always in accordance with the polarity of a compound.

No sorbent gave a mutual dependence between the constants n and $R_M{}^0$.

ИЗВОД

РЕТЕНЦИОНО ПОНАШАЊЕ НЕКИХ ДЕРИВАТА ЕСТРАДИОЛА НА АЛУМИНИЈУМ–ОКСИДУ У ХРОМАТОГРАФИЈИ НА НОРМАЛНИМ ФАЗАМА

МАРИЈАНА М. АЧАНСКИ

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Хроматографијом на танком слоју алуминијум-оксида испитано је ретенционо понашање деривата естрадиола. Такође је испитана и промена ретенције истих једињења са порастом запреминског удела поларније компоненте у бинарним покретним фазама. Установљена је линеарна зависност између ретенционих константи деривата естрадиола и логаритма запреминског удела поларније компоненте бинарних покретних фаза. Вредности нагиба и одсечака линеарне зависности дискутоване су у зависности од природе растворка и састава покретне фазе. Добијени резултати су упоређени са резултатима добијеним у ранијим испитивањима на силика–гелу.

(Примљено 24. септембра, ревидирано 8. новембра 2002)

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