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CORRELATION BETWEEN THE MOLECULAR STRUCTURE OF CARDIAC GLYCOSIDES, STEROID HORMONES AND CARBOHYDRATES AND THEIR RETENTION IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Cardiac glycoside molecules and other molecules can be thought as consisting of a number of functional groups that make individual average contributions to the overall retention. To evaluate these contributions it was necessary to solve a system of linear equations by the last-squares method for a number of cardiac glycosides and steroid hormones, the retention volumes of which were determined under standard conditions. Experimental values of the logarithm of retention volumes are compared with calculated values, $\ln V_{R'} = \Sigma n_i a_i$, where $a_i = \ln (V_{R'})_i$ is the contribution to the retention of group i and n_i is the number of functional groups in a molecule. Such a comparison shows that these values are in good agreement and the method may be used to evaluate the retentions of cardiac glycosides, steroid hormones and carbohydrates.

The correlation of biological activity of cardiac glycosides and the logarithm of retention volumes is expressed by a linear equation, the coefficients of which have been determined. This equation shows that biological activity is related to the hydrophilic–hydrophobic properties of cardiac glycosides and consequently with the transport of these compounds to the receptor.

INTRODUCTION

High-performance liquid chromatography (HPLC) is widely used for the analysis of cardiac glycosides, steroid hormones and carbohydrates, which possess high biological activity[1–5]. The experimental data on the separation of these compounds by HPLC make it possible to determine their retention values on the basis of some general regularities.

The effects of a number of factors on the retention and separation of these compounds have been reported[3,6]. The retention of cardiac glycosides, steroid hormones and carbohydrates depends on the surface chemistry of the adsorbent, the eluent composition and the structure of the molecules being separated. For the cardiac glycoside cymarin it has been shown that the retention almost does not depend on the number of carbon atoms in the modifying group if this number exceeds 6–8.

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This applies to adsorbents with bonded *n*-alkyl and phenyl groups[6,7].

Studies on the retention of these compounds using adsorbents with the same surface chemistry and the same eluent make it possible to elucidate the effect of the structure of the separated molecules on their retention.

No correlation has been observed between the molecular mass of cardiac glycosides and their retention in reversed-phase HPLC[3], whereas the hydrophobic properties of glycone and aglycone have been shown to be correlated with the retention volume. It has been shown that in many instances aglycone properties are the most important for retention in HPLC. It has been also shown that not all functional groups (*e.g.*, hydroxy groups) make the same contribution to retention, but their contribution depends on the position of the hydroxy group in cardiac glycoside molecules. Such regularities can be used for the separation and analysis of steroids. A general approach to the determination of retention in HPLC on the basis of molecular structure is the most important problem.

In this work, such an attempt has been made for non-ionogenic molecules using cardiac glycosides, steroid hormones and carbohydrates as a model.

EXPERIMENTAL

The retention volumes of cardiac glycosides and steroid hormones were determined using a Spectra-Physics 3500B liquid chromatograph with a Model 770 UV–VIS detector on 125 × 4.8 mm I.D. column filled with LiChrosorb Si 60 and LiChrosorb Si 100 silica gel with bonded diphenylsilyl groups. The silica gels were chemically modified by the methods described previously[8,9]. The surface concentrations of bonded diphenylsilyl groups of the adsorbents were similar. The retention volumes were determined at 50°C using ethanol–water (35:65) as the eluent. The above conditions were considered to be standard.

RESULTS AND DISCUSSION

Cardiac glycosides consist of a steroid part and a sugar residue. Hence the retention of their molecules can be related to aglycone properties for glycosides with different aglycones or, in the case of the same aglycone, to those of glycone, as mentioned previously[3]. However, it seems to be more correct to consider a molecule as a whole. Each functional group can be assumed to make its own contribution to the retention of a molecule, *i.e.*, $\ln V_{R'} = \Sigma a_i n_i$, where a_i is the contribution to retention of functional group i and n_i is the number of functional groups of a given type. The coefficient a_i is the the logarithm of the retention volume of group i, $a_i = \ln (V_{R'})_i$, with the assumption that each group is independently adsorbed, *i.e.*, additivity of interaction with the surface is fulfilled. Such an approach resembles the description of adsorption from the gaseous phase using atom–atom potentials. This is a rough approximation because in large molecules such groups can be located at different distances from the surface and therefore the energy of their interaction with the surface will differ. During the adsorption from solutions when the interaction of an adsorbed molecule with the eluent is of great importance, the difference in adsorption energy becomes smaller, although for some groups the energy of interaction is different from the average energy of interaction of similar groups with the adsorbent.

If it is shown experimentally that one group differs greatly from other similar groups then it should be considered as a separate group with its own contribution to retention. The molecules of cardiac glycosides and also steroid hormones and carbohydrates can be assumed to consist of –OH, –O–, >C=O, –C(H)=O, –C< and $-CH_n$ (n = 1–3) groups.

To calculate the contribution of all groups to retention under some standard conditions, it is necessary to determine the retention volumes for a number of compounds possessing different amounts of such groups and to use the least-squares method to obtain a_i values which can be used for the evaluation of retention volumes on the basis of the empirical formula of the compound.

To obtain a_i coefficients the retention volumes of cardiac glycosides were measured on LiChrosorb Si 60 columns with modifying diphenylsilyl groups and the previously obtained data[9] on the retention of steroid hormones on LiChrosorb Si 100 columns with modifying diphenylsilyl groups were used. As the specific surface areas of modified adsorbents differ from each other, in order to obtain comparable data the retention value V_s (μl/m^2) should be introduced, with $V_s = V_a/S$, where V_a is the retention volume per gram of adsorbent (ml/g) and S is the specific surface area (m^2/g).

Table I shows the retention values (ln V_s) of cardiac glycosides and Table II shows those of steroid hormones, making it possible to correlate the retention values with molecular structure.

The values of a_i were obtained by solving a system of linear equations using the least-squares method for the cardiac glycosides and steroid hormones mentioned in Tables I and II.

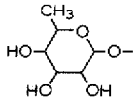
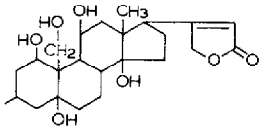
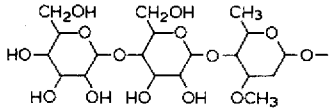
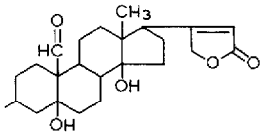
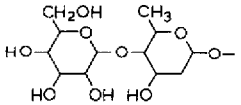
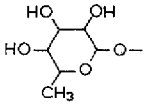
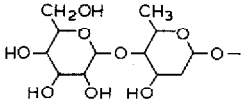
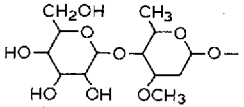
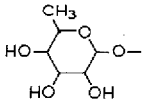
The calculated a_i values are presented in Table III. The following three approximations were used: (I) all OH groups are similar to each other as well as –C<, >C=, –C≡ and $-CH_3$, $-CH_2-$, >C–H, =CH–, ≡CH groups, respectively; (II) all OH groups are similar except for $-OH(C_{12})$ and $-OH(C_{16})$ as well as –C<, >C=, –C≡ and $-CH_3$, $-CH_2-$, >CH, =CH–, ≡CH groups, respectively; (III) all OH groups are similar except for $-OH(C_{12})$ and $-OH(C_{16})$ as well as all –C<, >C=, –C≡, $-CH_3$, $-CH_2-$, >CH, =CH– and ≡CH groups. As shown in Table III, the retention of steroids is decreased by all hydrophilic groups to a certain extent whereas $-CH_n$ increases the retention.

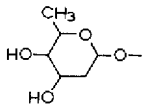
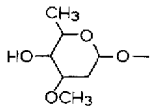
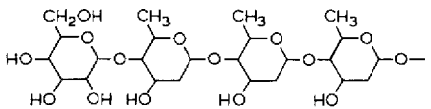
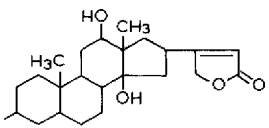
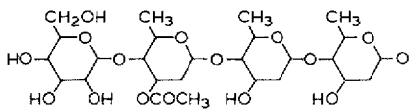
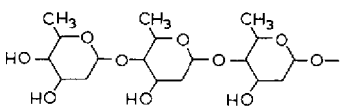
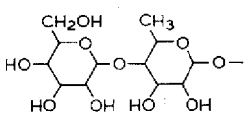
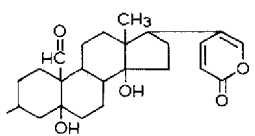
Correlation of experimental ln V_s values with those calculated by the additive scheme ($\Sigma\, a_i n_i$) is shown for three approximations in Fig. 1. The experimental and calculated values are shown to correlate well with correlation coefficients r = 0.954 for approximation I, r = 0.964 for approximation II and r = 0.964 for approximation III. According to Fig. 1 and Table III, the distinction of –OH groups in C_{12} and C_{16} compounds results in an increase in the correlation coefficient whereas it is not changed after combining –C<, >C=, –C≡ groups and $-CH_3$, $-CH_2-$, >CH, =CH– and ≡CH groups as similar groups. Hence, using a_i coefficients, it is fairly easy to calculate retention volumes.

Using a_i values for functional groups and the additive scheme, it is possible to calculate the contribution of aglycone and glycone in the retention of various glycosides. Then the logarithm of the retention volume of cardiac glycoside can be presented as $\ln V_s = \ln V_{s\,gl} + \ln V_{s\,agl}$. According to the values of $\ln V_{s\,gl}$ and $\ln V_{s\,agl}$ in Table I, the main contribution to retention is made by aglycone. As for

TABLE I

RETENTION OF CARDIAC GLYCOSIDES ON SILICA GEL WITH BONDED DIPHENYLSILYL GROUPS USING WATER–ETHANOL ELUENT (65:35) AT 50°C (ln V_s) AND CALCULATED CONTRIBUTION OF GLYCONE (ln $V_{s\ gl}$) AND AGLYCONE (ln $V_{s\ agl}$) TO RETENTION

Cardiac glycoside	*Glycone*	*ln ($V_{s\ gl}$, $\mu l/m^2$)*	*Aglycone*	*ln ($V_{s\ agl}$, $\mu l/m^2$)*	*ln (V_s, $\mu l/m^2$)*
G-strophanthin		−0.7942		1.9864	0.6215
K-strophanthoside		−1.1729			1.6952
Eryzimoside		−0.9924			1.8635
Convallatoxin		−0.7942			1.9086
Olitoriside		−0.9924			1.9477
K-strophanthin-β		−0.3781		2.7270	2.0673
Desglucocheirotoxin		−0.7942			2.1096

Strophanthidin	HO–	−0.5960			2.1823
Eryzimin		−0.1982			2.4508
Strophanthidin acetate	CH_3COO–	−0.1504			2.5377
Cymarin		0.4161			2.8976
Desacetyl-lanatoside C		−0.1968			2.7483
Lanatoside C		0.2488		2.9017	3.2519
Digoxin		0.5974			3.3605
Corelborin-π		−1.5884		2.9663	1.7898

(*continued on p. 128*)

TABLE I (*continued*)

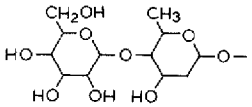
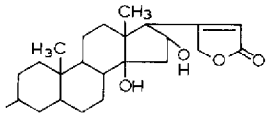
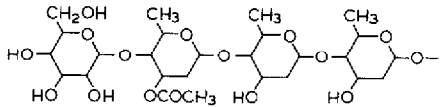
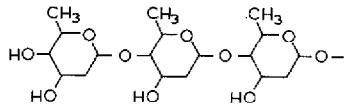
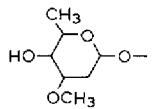
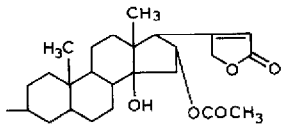
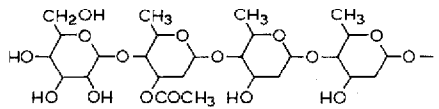
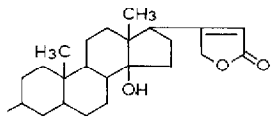
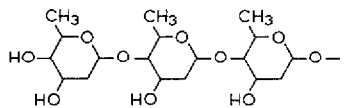
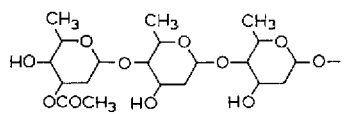
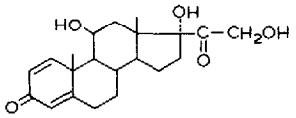
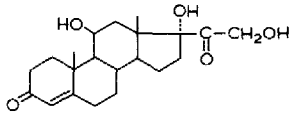
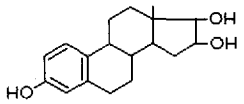
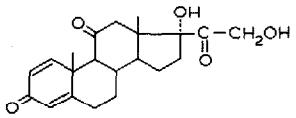
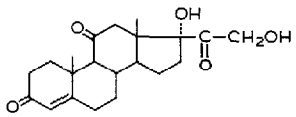
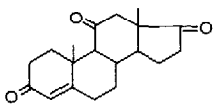
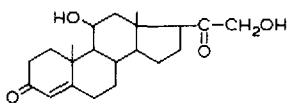
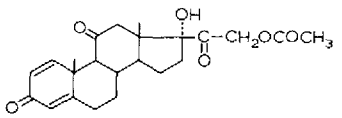
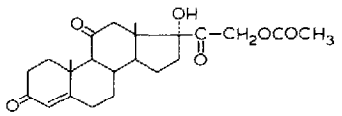
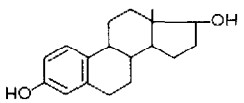
Cardiac glycoside	*Glycone*	*ln ($V_{s\ gl}$, $\mu l/m^2$)*	*Aglycone*	*ln ($V_{s\ agl}$, $\mu l/m^2$)*	*ln (V_s, $\mu l/m^2$)*
Glucogitoroside		−0.9924			2.3275
Lanatoside B		0.2488		3.5407	3.9455
Gitoxin		0.5974			4.1155
Oleandrin		0.4161		4.2017	4.0109
Lanatoside A		0.2488			4.7836
Digitoxin		0.5974		4.3521	4.8866
Acetyldigitoxin		1.0430			5.4660

TABLE II

RETENTION OF STEROID HORMONES ON SILICA GEL WITH BONDED DIPHENYL SILYL GROUPS USING WATER–ETHANOL ELUENT (65:35) AT 35°C

No.	*Steroid hormone*	*Formula*	*ln (V_s, $\mu l/m^2$)*
1	Prednisolone		2.4132
2	Hydrocortisone		2.4406
3	Estriol		2.4706
4	Prednisone		2.7233
5	Cortisone		2.7562
6	Adrenosterone		2.9014
7	Corticosterone		3.1544
8	Prednisone acetate		3.2064
9	Cortisone acetate		3.3028
10	Estradiol		3.5721

(*continued on p. 130*)

TABLE II (*continued*)

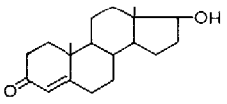
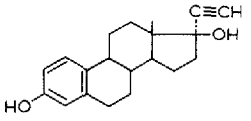
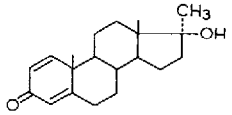
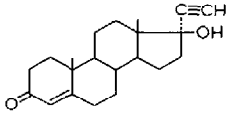
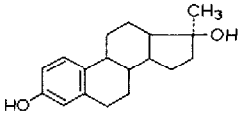
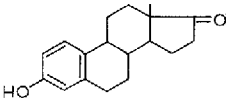
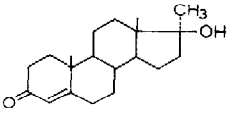
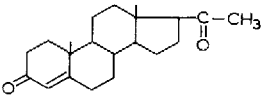
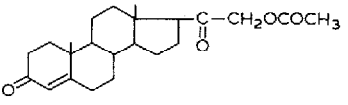
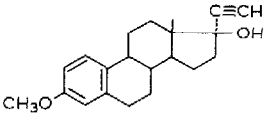
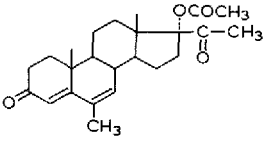
No.	*Steroid hormone*	*Formula*	*ln (V_s, $\mu l/m^2$)*
11	Testosterone		3.6304
12	Ethynylestradiol		3.6612
13	Methanedrosteonolone		3.7131
14	Pregnine		3.7177
15	Methylestradiol		3.7235
16	Estrone		3.7568
17	Methyltestosterone		3.8126
18	Progesterone		4.5165
19	Desoxycorticosterone acetate		4.5601
20	Mestranol		4.7163
21	Megestrol acetate		4.8651

TABLE III

VALUES OF a_i FOR VARIOUS FUNCTIONAL GROUPS OF CARDIAC GLYCOSIDES AND STEROID HORMONES

Adsorbent, silica gel with bonded diphenylsilyl groups; eluent, water–ethanol (65:35); temperature, 50°C.

Functional group	a_i		
	I	*II*	*III*
–OH (C_{12})		−1.4504	−1.4603
–OH (C_{16})		−0.8114	−0.8188
–OH	−0.6247	−0.5960	−0.6055
–O–	−0.1879	−0.2210	−0.2315
>C=O	−0.1317	−0.1687	−0.1598
	−0.6805	−0.8081	−0.7854
–C≡	0.3187	0.2576	
$-CH_n$	0.2211	0.2393	0.2444

glycones they can both increase ($\ln V_{s\,gl} > 0$) and decrease ($\ln V_{s\,gl} < 0$) the retention, depending on their structure.

Carbohydrates are more frequently separated on adsorbents with amino groups[4,6], but it is possible to determine the retention volumes of carbohydrate molecules under standard conditions on the basis of the contributions of various functional groups to retention. Table IV shows the calculated values of $\ln V_s$ for some sugars incorporated into the molecules of cardiac glycosides. It is impossible to define the stereoisomeric molecules of sugars using such a calculation, although different $\ln V_s$ values can be obtained for pentoses, hexoses and sugar esters. The retention volumes of given sugars are not high and they cannot be separated under such standard conditions, although the relative retention volumes can be calculated. As shown in Table IV, for cymarose and olcandrose $\ln V_s > 0$ and their introduction into a molecule of glycoside results in an increase in retention. The introduction of mono-, di- and trisaccharides of digitoxose into a molecule would result in an increase in retention volume, whereas introduction into a molecule of cardiac glycosides of glucose, rhamnose and gulomethylose would lead to a decrease in retention volume.

This explains the dependence of the retention of digitoxigenin, digoxigenin and strophanthidin glycosides on a number of monosaccharide residues shown in Fig. 2 and Table V. Fig. 2 shows the dependence of the logarithms of the capacity factors of some cardiac glycosides on a number of monosaccharides. The values of $\ln k'$ for digoxigenin and digitoxigenin glycosides were calculated on the basis of data from ref. 10. The calculated data in Table V illustrate the reason for the different dependences of capacity factors ($\ln k'$) on glycone length for glucose- and digitoxose-containing glycones.

Hence with the additive scheme it is possible to calculate the retention volumes of non-ionogenic compounds on the basis of their structural formulae and the contributions to retention of various functional groups. More detailed separations can be carried out with respect to the differences in molecular geometry as a whole.

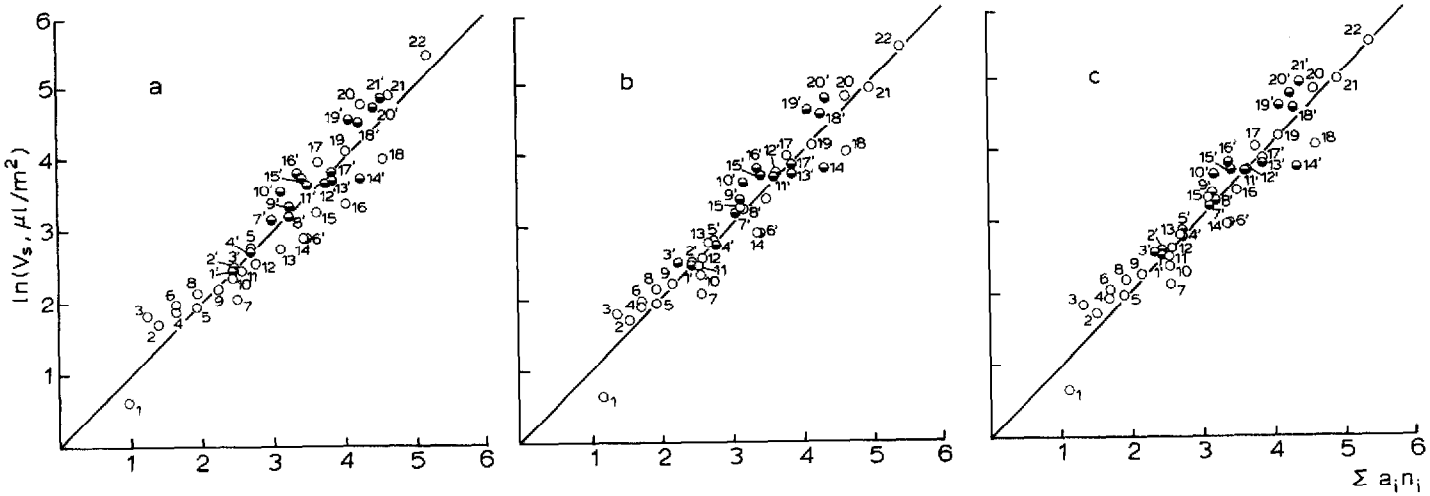
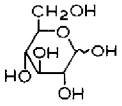
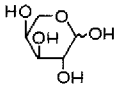
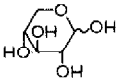
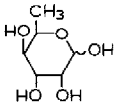
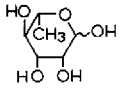
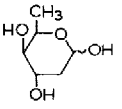
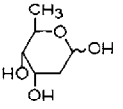
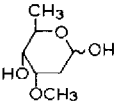
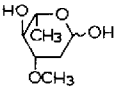


Fig. 1. Correlation between the experimental values (ln V_s) of cardiac glycosides (○) and steroid hormones (◑) and the calculated values ($\Sigma a_i n_i$) according to Table III: (a) I; (b) II; (c) III. The numbers correspond to the compounds for cardiac glycosides: 1 = G-strophanthin; 2 = K-strophanthoside; 3 = corelborine-π; 4 = eryzimoside; 5 = convallatoxin; 6 = olitoriside; 7 = K-strophanthin-β; 8 = desglucocheirotoxin; 9 = strophanthidin; 10 = glucogitoroside; 11 = eryzimin; 12 = strophanthidin acetate; 13 = desacetyl lanatoside C; 14 = cymarin; 15 = lanatoside C; 16 = digoxin; 17 = lanatoside B; 18 = oleandrin; 19 = gitoxin; 20 = lanatoside A; 21 = digitoxin; 22 = acetyl digitoxin; and for steroid hormones in Table II.

TABLE IV

CALCULATED VALUES OF ln V_s UNDER STANDARD CONDITIONS FOR SOME MONOSACCHARIDES INCORPORATED INTO CARDIAC GLYCOSIDES

Sugar	*Formula*	*ln (V_s, $\mu l/m^2$)*
D-Glucose		−1.7652
L-Arabinose		−1.4085
D-Xylose		
D-Gulomethylose		−1.1692
L-Rhamnose		
D-Bovinose		−0.5732
D-Digitoxose		
D-Cymarose		0.0411
L-Oleandrose		

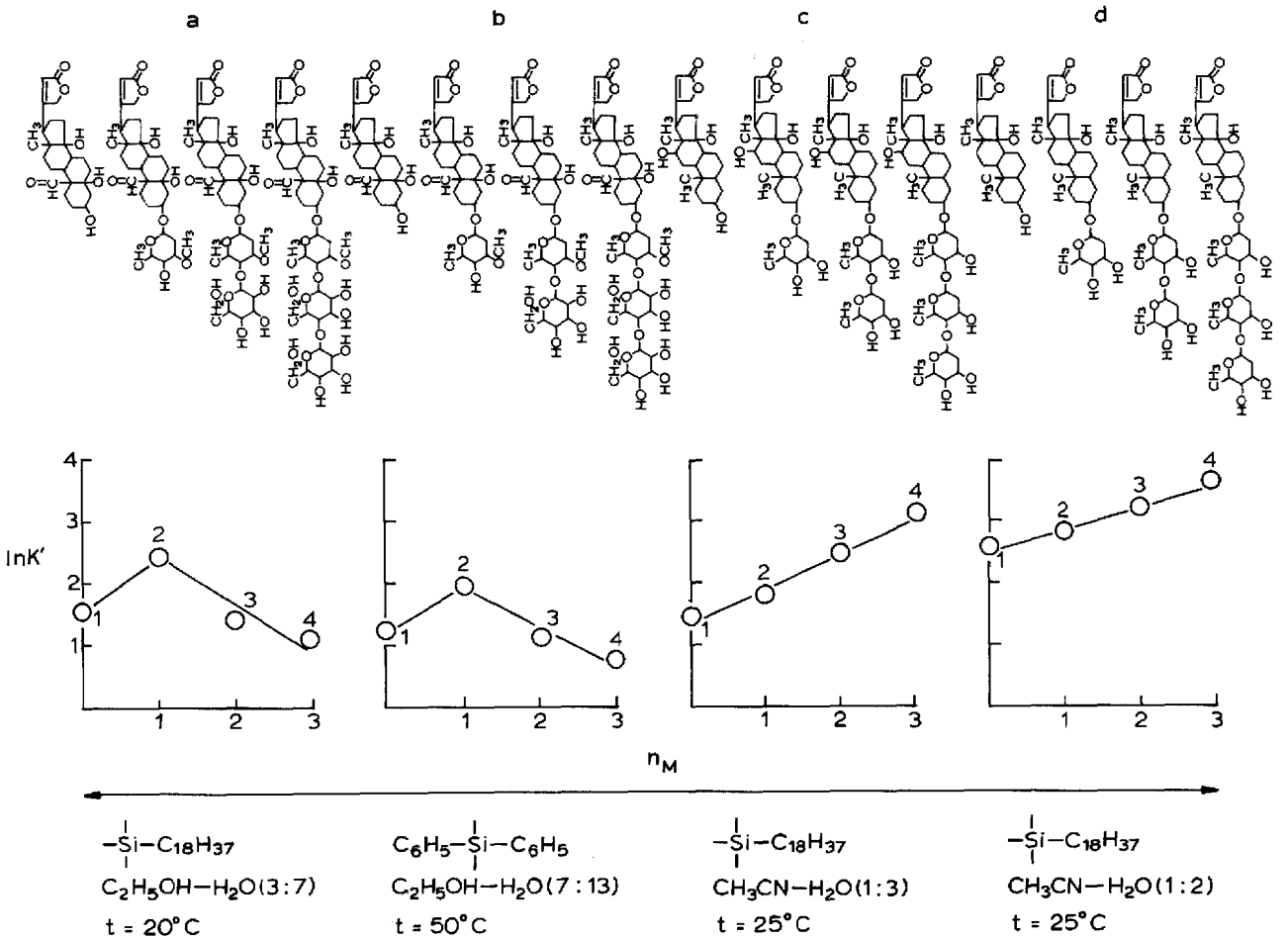


Fig. 2. Dependence of ln k' of cardiac glycosides on the number of monosaccharide groups in glycone. (a) and (b) 1 = strophanthidin; 2 = cymarin; 3 = K-strophanthin-β; 4 = K-strophanthoside; (c) 1 = digoxigenin; 2 = digoxigenin monodigitoxoside; 3 = digoxigenin bisdigitoxoside; 4 = digoxin; (d) 1 = digitoxigenin; 2 = digitoxigenin monodigitoxoside; 3 = digitoxigenin bisdigitoxoside; 4 = digitoxin.

TABLE V

CALCULATED VALUES OF ln V_s FOR SOME AGLYCONES AND GLYCOSIDES UNDER STANDARD CONDITIONS

Compound	*ln (V_s, $\mu l/m^2$)*
Oubagenin	1.3904
Strophanthidin	2.1310
Cymarin	3.1431
K-strophanthin-β	2.3109
K-strophanthoside	1.5547
Digoxigenin	2.3057
Digoxigenin monodigitoxoside	2.7035
Digoxigenin bisdigitoxoside	3.1013
Digoxin	3.4991
Hellebrigenin	2.3703
Gitoxigenin	2.9447
Oleandrigenin	3.6057
Digitoxigenin	3.7561
Digitoxigenin monodigitoxoside	4.1539
Digitoxigenin bisdigitoxoside	4.5517
Digitoxin	4.9495

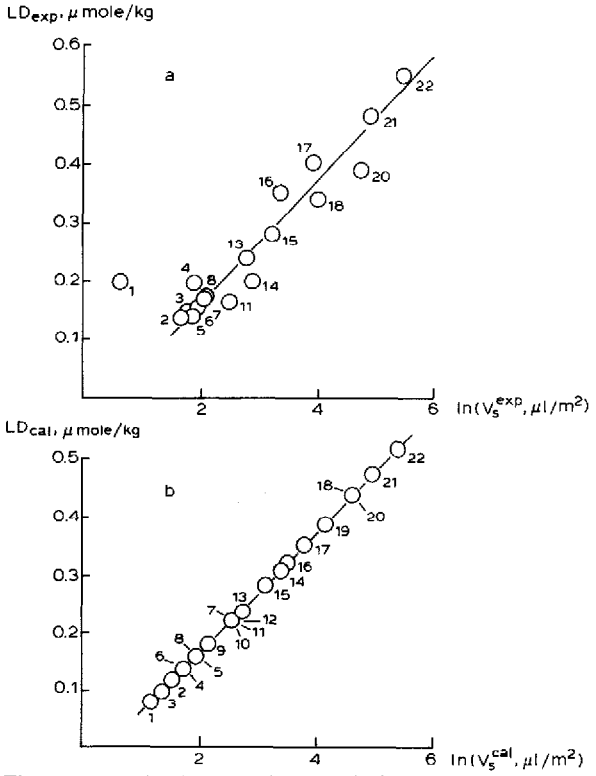


Fig. 3. Correlation between the biological activity of cardiac glycosides and their retention (ln V_s) on silica gel with bonded diphenylsilyl groups from water–ethanol solution: (a) experimental values of ln V_s and *LD*; (b) calculated values. The numbers correspond to those for cardiac glycosides in Fig. 1.

In addition to the determination of the retention volumes of cardiac glycosides, the additive scheme can also be used for the determination of biological activity.

The retention volumes of cardiac glycosides were compared with lethal doses[3] using published data[11] which reflect the biological activity of cardiac glycosides. It seems more advisable to correlate the lethal dose (μmole/kg) with the logarithm of retention volume. In this instance the dependence of lethal dose (LD) on the logarithm of retention volume can be represented by the following equation:

$$LD = 0.104 \ln V_s - 0.044$$

with a correlation coefficient $r = 0.964$ for $n = 17$.

Fig. 3 shows such a dependence, (a) obtained experimentally and (b) calculated. The retention volumes were calculated by the additive scheme and LD values were calculated using the equation relating the biological activity to the retention volume. Fig. 3 shows a good correlation between the calculated and experimental data.

CONCLUSION

The additive scheme can be used for determination of retention volumes in the reversed-phase HPLC of non-ionogenic compounds on the basis of contributions to the retention of individual functional groups and the number of groups in a molecule. Possibly the method can also be used for determination of the biological activity of cardiac glycosides.

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