Journal of Chromatography, 248 (1982) 49–62
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,086

CORRELATION BETWEEN THE RETENTION OF CARDIAC GLYCOSIDES IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH A DIPHENYLSILYL STATIONARY PHASE, THE STRUCTURE OF THEIR MOLECULES AND THEIR BIOLOGICAL ACTIVITY

V. Ya. DAVYDOV, M. ELIZALDE GONZALEZ and A. V. KISELEV*
Chemistry Department, Lomonosov State University of Moscow, 117234 Moscow (U.S.S.R.)
(First received March 30th, 1982; revised manuscript received June 3rd, 1982)

SUMMARY

The separation of mixtures of cardiac glycosides by reversed-phase high-performance liquid chromatography on silica gel with chemically grafted diphenylsilyl groups using water–ethanol as the eluent was carried out. It is shown that the configuration and conformation of the glycoside molecules, and the hydrophilic properties of their aglycones and glycones, influence the separation. The hydrophilic properties of the aglycones are more important than those of the glycones. The glycosides with more hydrophilic aglycones have higher biological activity. This is probably related to the easier transport of these glycosides to the receptor.

INTRODUCTION

The investigation of the correlation between molecular structure and chromatographic retention is of great interest, as it permits the mechanisms of intermolecular interactions in adsorption from solution to be established and the reverse chromatographic problem, *i.e.*, to obtain information about molecular structure from chromatographic data, to be solved. Such problems have been solved in simpler cases in gas chromatography[1–5]. For the development of such an approach using liquid chromatography, it is first necessary to obtain reproducible experimental results, for which we need to take into account more complicated intermolecular interactions in the system adsorbent–compound under investigation–eluent (often multi-component).

In previous papers[6–9] we attempted to find a correlation between the structure of cardiac glycoside molecules and their retention on hydrophobic silica gel when eluting with water–ethanol solutions. It was found that the retention volume does not depend directly on the molecular weight of the glycosides, but on the hydrophilic properties of their molecules. It was also found that the retention volume of glycosides depends on the configuration of their molecules.

Cardiac glycosides, mainly digitalis glycosides, have been studied by high-performance liquid chromatography (HPLC) by many workers[10–26]. We have considered the HPLC not only of digitalis glycosides but also of other types of cardiac

0021-9673/82/0000–0000/$02.75

glycosides without derivatization on the silica gel surface with attached rigid diphenylsilyl groups, while silica gels with octadecylsilyl (ODS) groups having conformational mobility were usually used.

The correlation between the chemical structure and chromatographic behaviour of cardiac glycosides was investigated earlier by paper (PC) and thin-layer chromatography (TLC) (see, for example, refs. 27–33). This correlation depends on the type of adsorbent and on the composition of the eluent. In PC and TLC, hydrophilic adsorbents and multi-component solvents were usually used.

The correlation between biological activity and chromatographic behaviour (QSAR studies) on the basis of hydrophobic, electronic and structural properties of biologically active molecules is important[34,35]. However, these properties are difficult to determine for such complicated molecules as glycosides. The retention volume depends on many types of intermolecular interactions and on the properties of adsorbent, eluent and solute. Therefore, the correlation between the retention volume itself and the biological activity of cardiac glycosides is useful.

In order to find a more general relationship between the retention and molecular structure of glycosides, in this work the retention volumes of different glycosides on silica gel with chemically grafted diphenylsilyl groups have been determined and the separation of glycoside molecules, which differ only in the molecular configuration, has been investigated. We have also attempted to find a correlation between the chromatographic properties of glycosides and their biological activity.

EXPERIMENTAL

The cardiac glycosides investigated are listed in Table I. The biological activities were taken from ref. 36.

A Spectra-Physics 3500B liquid chromatograph was used with a UV–VIS 700 detector at 220 nm. Stainless-steel columns (125 mm × 4.8 mm I.D.) were packed with silica gel modified with diphenyldichlorosilane[8,9], LiChrosorb Si 60 or LiChrosorb Si 100 of particle size 5 and 7 μm, respectively. In contrast to *n*-alkylsilyl groups, the attached diphenylsilyl groups do not exhibit conformational transformations in polar eluents (phenyl groups are rigid and can undergo only restricted rotation around the silicon–carbon bonds, producing an adsorbent with a considerably more uniform surface).

RESULTS AND DISCUSSION

The isomeric cardiac glycosides convallatoxin and desglucocheirotoxin have the same aglycone, strophanthidin, and the monosaccharide glycones have L-rhamnose and D-gulomethylose, respectively. They possess an identical number of hydroxyl groups. The structural difference between these isomers is only the different saccharide conformation and their bonds with the aglycone, which produces a relatively small difference in the molecular configuration. However, this difference is enough for the separation of these isomers on the silica surface modified with diphenylsilyl groups. Fig. 1 shows the separation of convallatoxin and desglucocheirotoxin on LiChrosorb Si 100 (specific surface area, $s = 285\ m^2/g$) with chemically grafted diphenylsilyl groups using water–ethanol as the eluent. The increase in the ethanol content of

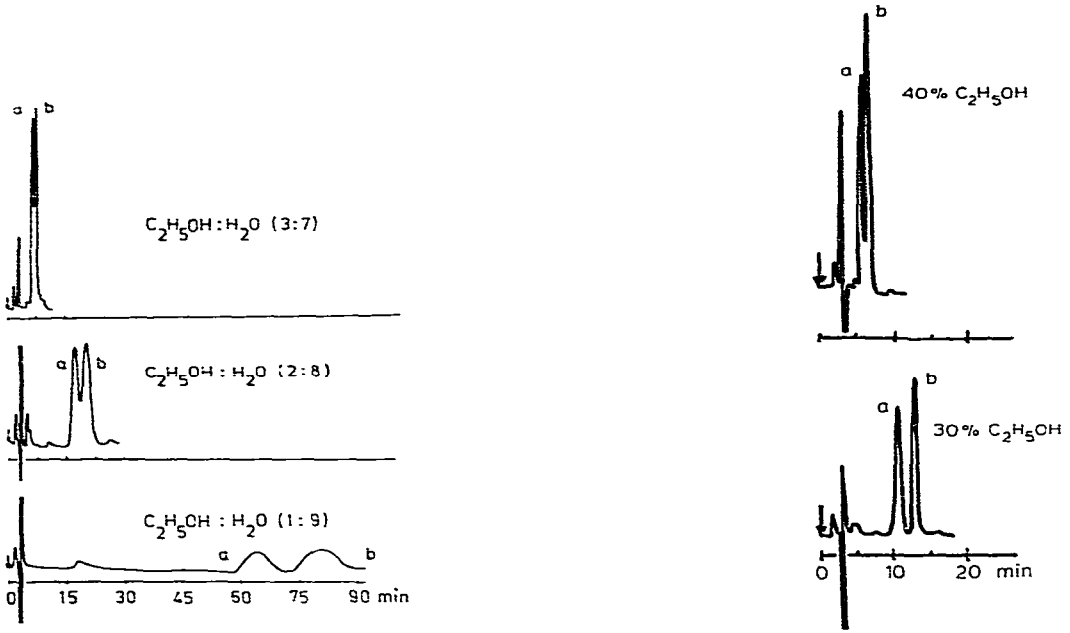


Fig. 1. Separation of a mixture of convallatoxin (a) and desglucocheirotoxin (b) on LiChrosorb Si 100 (7 μm) with diphenylsilyl groups grafted to the surface. Water–ethanol eluent composition as indicated. Column, 12.5 cm × 4.8 mm I.D.; flow-rate, 0.7 cm^3/min; temperature, 30°C.

Fig. 2. Separation of a mixture of convallatoxin (a) and desglucocheirotoxin (b) on LiChrosorb Si 60 (5 μm) with attached diphenylsilyl groups from water–ethanol eluent of different compositions as indicated. Column as in Fig. 1. Flow-rate, 0.65 cm^3/min; temperature, 40°C.

the eluent improves the separation, but does not lead to complete separation. The use of LiChrosorb Si 60, which has a larger specific surface area (s = 500 m^2/g), with attached diphenylsilyl groups made possible their complete separation (Fig. 2). It can be clearly seen that owing to the bond in strophanthidin-3β-O-α-L-rhamnose and the C1 conformation of L-rhamnose, the convallatoxin molecule as a whole is more curved than the desglucocheirotoxin molecule, for which the bond in strophanthidin-3β-O-β-D-gulomethylose and the 1C conformation of the D-gulomethylose form a flatter molecule.

Fig. 3 shows the dependence of the retention volumes of these two isomers on the water–ethanol eluent composition. With a decrease in the ethanol content the retention volume for both glycosides sharply increases, but the difference in the specific retention volumes, $V_{m,1}$, *i.e.*, in the adsorption equilibrium constant (Henry's constant)[7], increases. It was interesting to determine the dependence of other thermodynamic characteristics of adsorption of convallatoxin and desglucocheirotoxin from water–ethanol on silica gel with attached diphenylsilyl groups on the eluent composition. For this purpose the retention volumes of these glycosides at different temperatures were measured. Fig. 4 shows the dependence of ln $V_{m,1}$ on reciprocal temperature for different compositions of the eluent. From these plots the changes in the differential enthalpy and entropy of adsorption from dilute solutions were evaluated.

Fig. 5 shows the dependence of the retention volume (Henry's constant), the

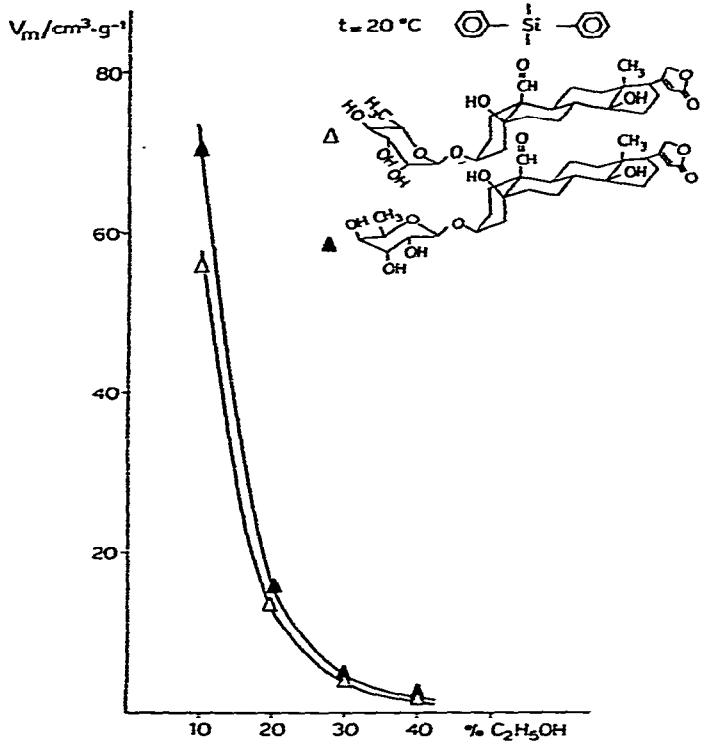


Fig. 3. Change in the retention volume of convallatoxin (a) and desglucocheirotoxin (b) on LiChrosorb Si 100 with attached diphenylsilyl groups on the ethanol concentration in the eluent. Temperature, 20°C.

differential enthalpy, $-\overline{\Delta H}_1$, and the standard differential entropy, $\overline{\Delta S}^\circ_1$, changes for the adsorption of these isomeric glycosides from water–ethanol on the eluent composition. The general character of the dependence of the thermodynamic characteristics of adsorption on the eluent composition is the same for these glycosides when adsorbed on the hydrophobic surface of modified silica gel from water–ethanol solution: a sharp decrease in the adsorption equilibrium constant occurs with increase in ethanol concentration in the eluent and a slow change in $-\overline{\Delta H}_1$ on increasing the ethanol concentration up to 20%, in comparison with the sharp decrease in $-\overline{\Delta H}_1$ at higher ethanol concentrations. A large increase in the adsorption equilibrium constant at lower ethanol concentrations is connected with the higher influence of the entropic factor, $-\overline{\Delta S}^\circ/R$.

For the elucidation of the relationship between the structure of the cardiac glycosides molecules and their retention, the seprations of mixtures of cardiac glycosides containing mono-, di-, tri- and tetrasaccharide glycones was carried out. Fig. 6 shows the separation of a mixture of glycosides containing the monosaccharide glycones G-strophanthin, convallatoxin, desglucocheirotoxin, erysimin, cymarin and oleandrin. The elution sequence of these glycosides from a column filled with silica gel with attached diphenylsilyl groups is determined by the number of hydrophilic groups in the glycoside molecule, as can be seen from the structural formulae. The largest

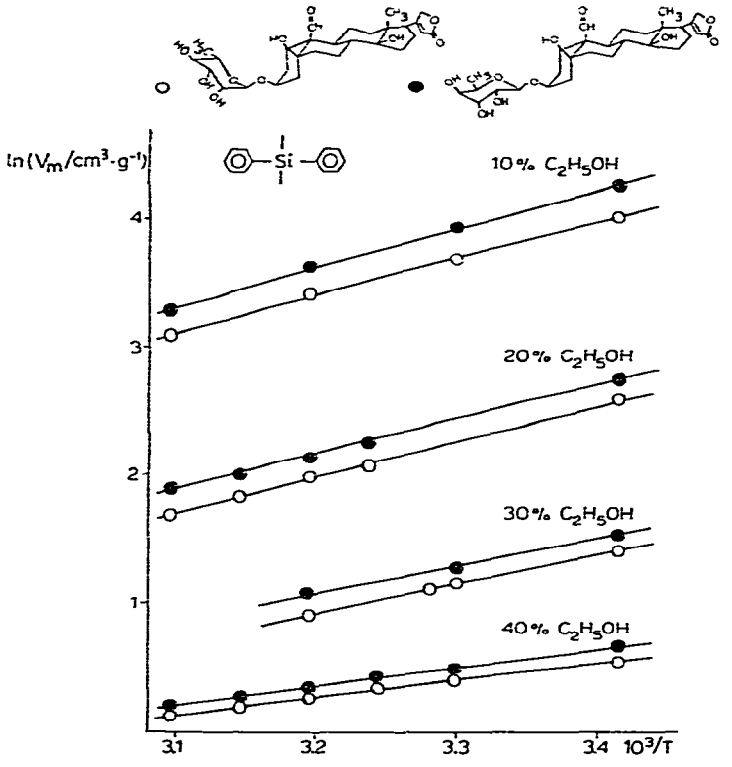


Fig. 4. Dependence of the logarithm of the retention volume on reciprocal temperature for convallatoxin and desglucocheirotoxin at different water–ethanol eluent compositions. Column filled with silica gel with diphenylsilyl groups grafted to the surface.

number of hydroxyl groups are present in the G-strophanthin molecule; there are five hydroxyl groups in the aglycone (oubagenin) and three in the glycone (L-rhamnose) of this molecule. As it is the most hydrophilic molecule in this mixture, G-strophanthin produces the strongest hydrogen bond type interaction with the water–ethanol eluent and the weakest interaction with the hydrophobic adsorbent surface. Therefore, G-strophanthin is eluted first. The convallatoxin molecule has the same glycone as G-strophantin (L-rhamnose) and a different aglycone, strophanthidin, which possesses only two hydroxyl groups. Thus convallatoxin is eluted second from this column. Third to be eluted is desglucocheirotoxin, which does not differ from its isomer convallatoxin in the number of hydroxyl groups, but has a flatter molecule (Fig. 1). The fourth glycoside, erysimin, differs from desglucocheirotoxin in the number of hydroxyl groups: its glycone (digitoxose) has only two hydroxyl groups. Then follows cymarine, whose glycone (cymarose) contains only one hydroxyl group. Last to be eluted is oleandrin, which also contains one hydroxyl group in its glycone (oleandrose), but in the aglycone (oleandrigenin) it has only one hydroxyl group.

Hence the larger the number of hydroxyl groups in the molecules of the glycosides in this mixture, the weaker is their retention on the silica gel with chemically grafted diphenylsilyl groups on the surface from water–ethanol solution.

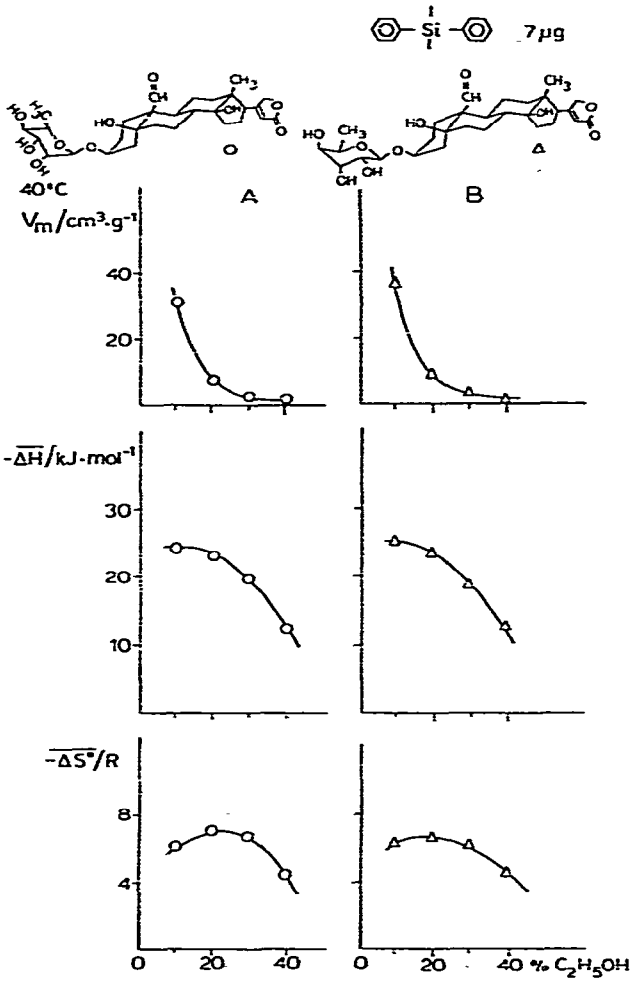


Fig. 5. Dependence of the specific retention volume, $V_{m,1}$, and the differential changes of enthalpy, $-\overline{\Delta H}_1$, and entropy, $-\overline{\Delta S}_1^\circ/R$, for the adsorption of convallatoxin (A) and desglucocheirotoxin (B) on Li-Chrosorb Si 100 with attached diphenylsilyl groups on composition of the water–ethanol eluent.

An analogous dependence of the retention of glycosides on the number of hydroxyl groups in the molecule is observed for glycosides with disaccharide glycones, such as for the mixture of corelborin-π, olitoriside and K-strophanthin-β. The aglycones of these three glycosides have an identical number of hydroxyl groups (two), and therefore a different number of hydroxyl groups in the glycone mainly determines the retention difference (Fig. 7). First to be eluted is corelborin-π (six hydroxyl groups in the glycone), second is olitoriside (five hydroxyl groups) and third is K-strophanthin-β (four hydroxyl groups).

By separating a mixture of glycosides containing a trisaccharide glycone, the same regularity is observed (Fig. 8): first to be eluted is the more hydrophilic glycoside K-strophanthoside (seven hydroxyl groups in the glycone and two in the aglycone),

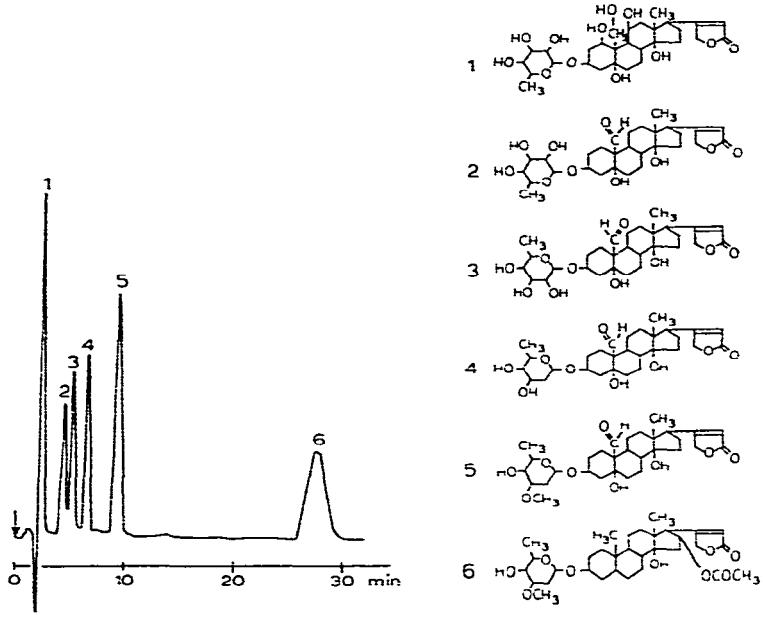


Fig. 6. Chromatogram of a mixture of cardiac glycosides on LiChrosorb Si 60 (5 μm) with attached diphenylsilyl groups from water–ethanol (65:35) eluent. Column, 12.5 cm × 4.8 mm I.D.; flow-rate, 0.9 cm^3/min; temperature, 50°C. Peaks: 1 = G-strophanthin; 2 = convallatoxin; 3 = desglucocheirotoxin; 4 = erysimin; 5 = cymarine; 6 = oleandrin.

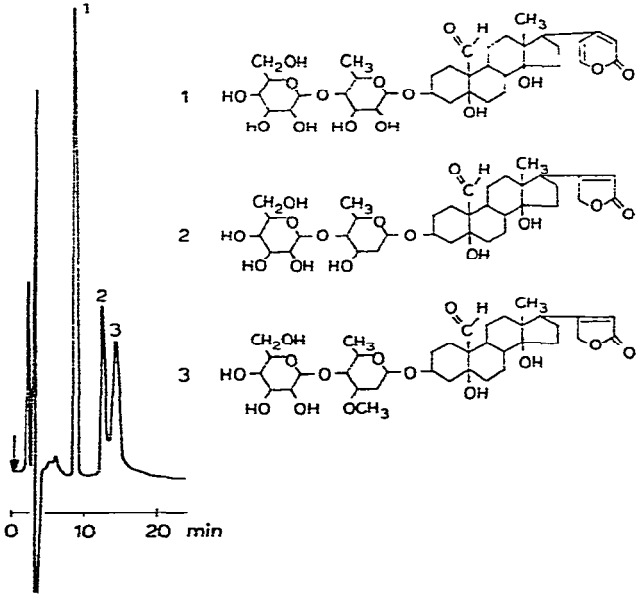


Fig. 7. Chromatogram of a mixture of cardiac glycosides on LiChrosorb Si 60 (5 μm) with attached diphenylsilyl groups from water–ethanol (70:30) eluent. Column as in Fig. 6. Flow-rate, 0.65 cm^3/min; temperature, 40°C. Peaks: 1 = Corelborin-π; 2 = olitoriside; 3 = K-strophanthin-β.

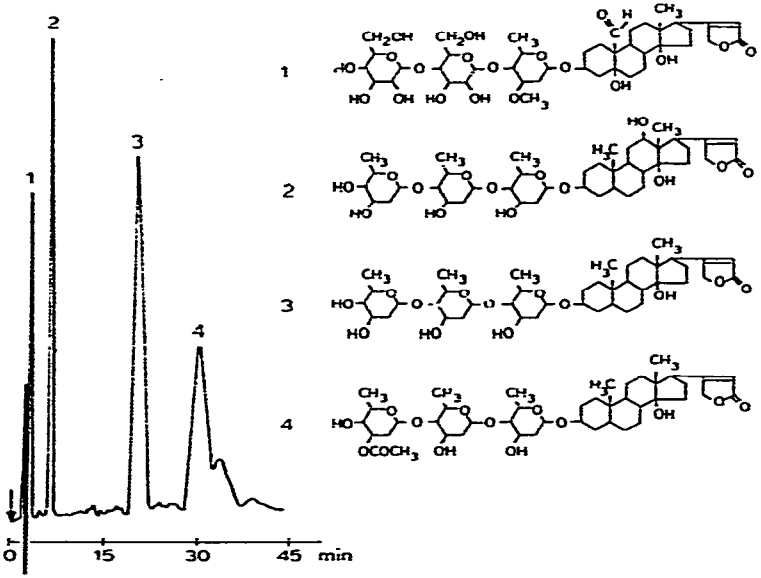


Fig. 8. Chromatogram of a mixture of cardiac glycosides on LiChrosorb Si 60 (5 μm) with attached diphenylsilyl groups from water–ethanol (60:40) eluent. Column as in Fig. 6. Flow-rate, 0.9 cm^3/min; temperature, 60°C. Peaks: 1 = K-strophanthoside; 2 = digoxin; 3 = digitoxin; 4 = acetyldigitoxin.

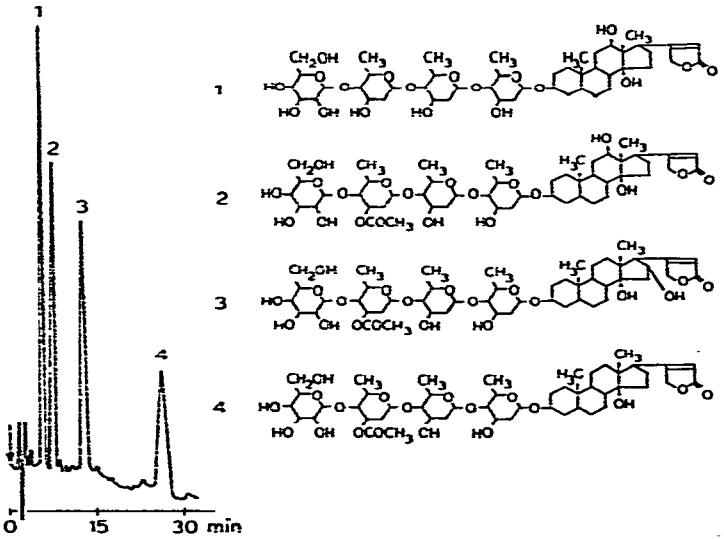


Fig. 9. Chromatogram of a mixture of cardiac glycosides on LiChrosorb Si 60 (5 μm) with attached diphenylsilyl groups from water–ethanol (60:40) eluent. Column as in Fig. 6. Flow-rate, 0.7 cm^3/min; temperature, 50°C. Peaks: 1 = Desacetyl-lanatoside C; 2 = lanatoside C; 3 = lanatoside B; 4 = lanatoside A.

second is digoxin (four and two hydroxyl groups, respectively), third is digitoxin (four and one hydroxyl groups, respectively) and last is acetyldigitoxin (three and one hydroxyl groups, respectively).

Fig. 9 shows the separation of a mixture of lanatosides having tetrasaccharide glycones: first to be eluted is desacetyl-lanatoside C (seven hydroxyl groups in the glycone and two in the aglycone), second is lanatoside C (six and two hydroxyl groups, respectively) and third is lanatoside B (also six and two hydroxyl groups, respectively). With lanatoside B, the hydroxyl group of the aglycone (gitoxigenine) in position C_{16} is screened, which results in a decrease in the hydrophilicity of the molecule[37], and therefore in the molecule of lanatoside B only seven hydroxyl groups remain "free". Last to be eluted is lanatoside A, which contains the aglycone digitoxigenin (six hydroxyl groups in the aglycone and one in the glycone).

The retention volumes of the 17 glycosides investigated were measured under identical conditions and are presented in Table I. Fig. 10 shows plots of ln $V_{m,1}$ against the number of hydroxyl groups, n_{OH}, in the whole cardiac glycoside molecules for each of these four mixtures containing mono-, di-, tri- and tetrasaccharide glycones. Fig. 10 shows that an increase in the number of hydroxyl groups in the molecules of cardiac glycosides produces a decrease in the retention volume but in a different manner for each mixture.

By grouping the glycosides with identical aglycones into families and arranging the aglycones so that their hydrophilic properties increase, *i.e.*, the number of all hydrophilic groups, it is possible to arrange the cardiac glycosides within each family according to the increase in the number of hydroxyl groups in the glycone. This permits a comparison of the change in the ln $V_{m,1}$ values with changes in the hydrophilic characteristics of the aglycone and of the whole glycoside molecule, although there is some uncertainty in the determination of the hydrophilic properties of parts of the molecule and the hydrophilic properties of the molecule as a whole. Fig. 11 shows this comparison. The glycosides are arranged in sequence according to the increase in the hydrophilic properties of the aglycone (by considering the increment in

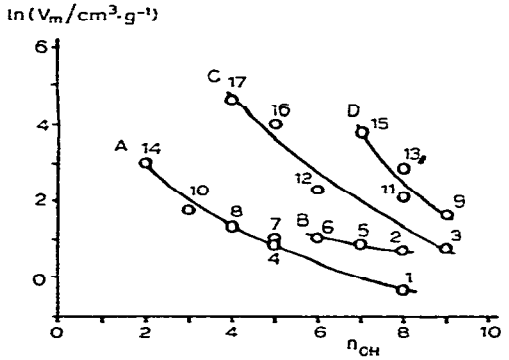
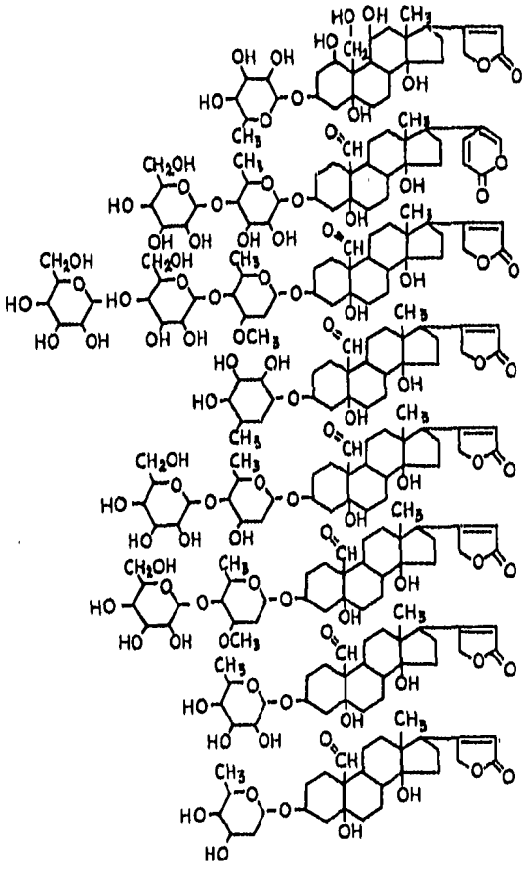
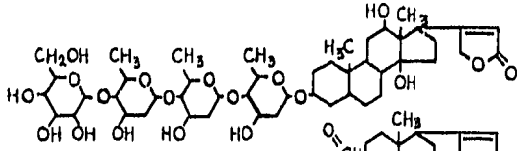
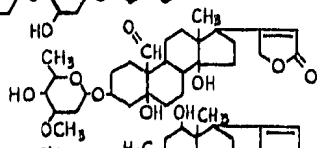
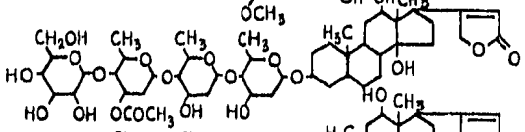
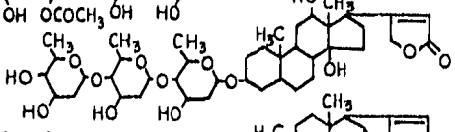
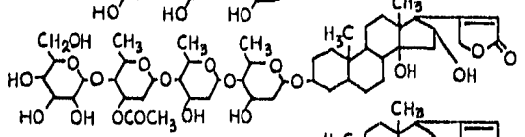
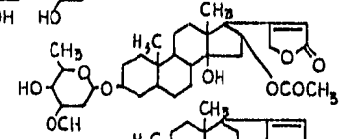
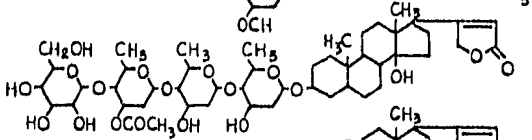
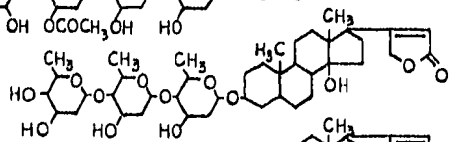
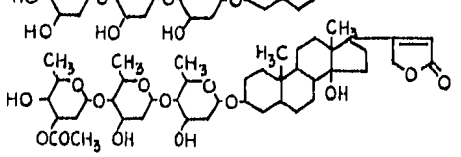


Fig. 10. Dependence of the logarithm of the retention volume on the number of hydroxyl groups in the glycoside molecule for the families of cardiac glycosides containing (A) mono-, (B) di-, (C) tri- and (D) tetrasaccharide glycones. The values of $V_{m,1}$ were determined on LiChrosorb Si 60 (5 μm) with attached diphenylsilyl groups from water–ethanol (65:35) eluent at 50°C. Numbers indicate the corresponding glycosides in Table I.

TABLE I

STRUCTURAL FORMULAE OF CARDIAC GLYCOSIDES, MOLECULAR WEIGHTS, VALUES OF HENRY'S CONSTANT, $K_1 = V_{m,1}$, FOR THE ADSORPTION OF GLYCOSIDES ON SILICA GEL (LICHROSORB Si 60, 5 μm) MODIFIED WITH DIPHENYLDICHLOROSILANE FROM WATER–ETHANOL (65:35) ELUENT AT 50°C, AND VALUES OF BIOLOGICAL ACTIVITY, LD (REF. 36)

No.	*Cardiac glycoside*	*Structural formula*	*Molecular weight*	$V_{m,1}^{50°C}$ *(cm³/g)*	*LD (mg/kg)*
1	G-strophanthin		584	0.72 ± 0.02	0.116
2	Corelborin-π		696	2.09 ± 0.09	0.104
3	K-strophanthoside		872	2.18 ± 0.09	0.126
4	Convallatoxin		550	2.36 ± 0.05	0.079
5	Oliorside		680	2.44 ± 0.02	0.103
6	K-strophanthin-β		694	2.83 ± 0.03	0.120
7	Desglucocheirotoxin		550	2.87 ± 0.03	0.096
8	Erysimin		534	3.90 ± 0.08	0.087

9	Desacetyl-lanatoside C		942	5.3 ± 0.2	0.228
10	Cymarin		548	6.0 ± 0.1	0.111
11	Lanatoside C		984	8.6 ± 0.4	0.280
12	Digoxin		780	10.4 ± 0.6	0.280
13	Lanatoside B		984	18.6 ± 0.9	0.403
14	Oleandrin		576	20.4 ± 0.1	0.197
15	Lanatoside A		967	46.5 ± 0.5	0.380
16	Digitoxin		764	58.3 ± 3.5	0.370
17	Acetyldigitoxin		806	107.2 ± 7.5	0.447

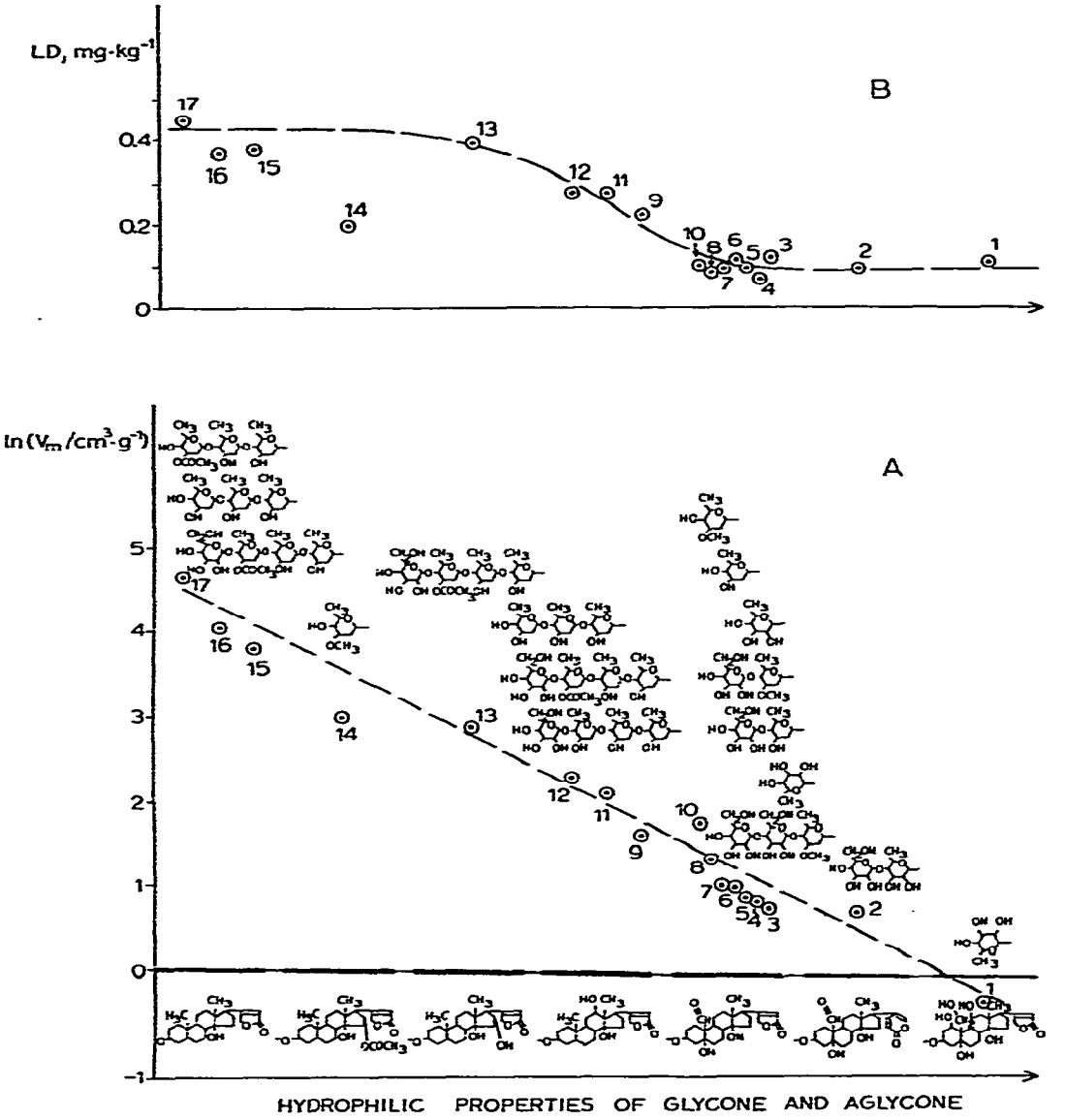


Fig. 11. Correlation of (A) ln $V_{m,1}$ and (B) biological activity, LD, of cardiac glycosides with the hydrophilic properties of their glycones and aglycones. Numbers indicate the corresponding glycosides in Table I.

the number of hydroxyl groups, their position in the steroid part and the presence of other groups such as aldehyde and acetoxy) and, for a given aglycone, in sequence according to the increase in the number of hydroxyl groups in the glycone. The $V_{m,1}$ values for the adsorption of cardiac glycosides on silica gel with attached diphenylsilyl groups from water–ethanol solution depend mainly on the hydrophilicity of the aglycones and to a smaller extent on the hydrophilicity of the glycones. Fig. 11 also

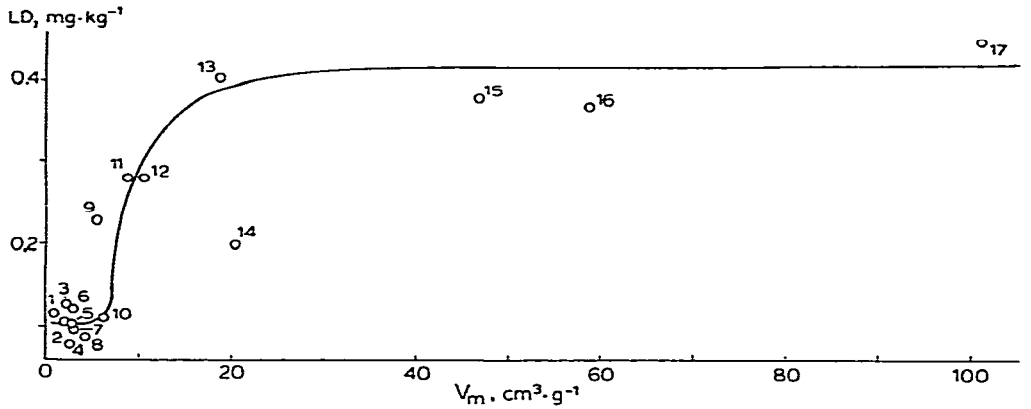


Fig. 12. Correlation between the biological activity, LD, of cardiac glycosides and their retention volumes, $V_{m,1}$, on the hydrophobic silica gel surface from water–ethanol eluent. Numbers indicate the corresponding glycosides in Table I.

shows the values of biological activity LD (ref. 36), expressed in milligrams of glycoside per kilogram of cat body-weight.

Fig. 12 shows the relationship between the biological activity of the cardiac glycosides and the retention volumes on adsorption on the hydrophobic silica gel surface containing diphenylsilyl groups. At values of $V_{m,1}$ up to *ca.* 6 cm^3/g, the biological activities of different glycosides are approximately the same and maximal (0.1 mg/kg). For $V_{m,1} > 18$ cm^3/g the cardiac glycosides manifest nearly identical biological activities but four times lower in comparison than those of the glycosides in the first group. Glycosides with $V_{m,1}$ between 6 and 18 cm^3/g have moderate biological activity.

The comparison of the biological activities of cardiac glycosides with their retention volumes on the hydrophobic surface of silica gel may demonstrate that the biological activity is connected with the transport of the glycosides to the corresponding receptor. The difference in the biological activities of different cardiac glycosides is also connected with the existence of the lactone ring in the steroid part of their molecules. The destruction of the lactone ring under the action of ultraviolet light, for example, results in the loss of biological activity[38].

REFERENCES

1 A. V. Kiselev, *Chromatographia*, 11 (1978) 691.
2 A. J. Grumadas, D. P. Poshkus and A. V. Kiselev, *J. Chem. Soc., Faraday Trans. 2*, 75 (1979) 1398; 78 (1982).
3 E. V. Kalashnikova, A. V. Kiselev, R. S. Petrova, K. D. Scherbakova and D. P. Poshkus, *Chromatographia*, 12 (1979) 799.
4 A. V. Kiselev and D. P. Poshkus, *15th Faraday Symposium: Chromatography, Equilibria and Kinetics*, Chemical Society, London, 1980, p. 13.
5 L. D. Dimitrov, A. V. Kiselev and R. S. Petrova, *Chromatographia*, 14 (1981) 107; 15 (1982) 245.
6 V. Ya. Davydov, A. V. Kiselev, I. V. Mironova and Yu. M. Sapojnikov, *Chromatographia*, 11 (1978) 591.
7 V. Ya. Davydov, A. V. Kiselev and Yu. M. Sapojnikov, *Chromatographia*, 13 (1980) 745.
8 V. Ya. Davydov, M. Elizalde Gonzalez, A. V. Kiselev and K. Lenda, *Chromatographia*, 14 (1981) 13.

9 V. Ya. Davydov, M. Elizalde Gonzalez and A. V. Kiselev, *J. Chromatogr.*, 204 (1981) 293.
10 R. A. Henry and J. A. Schmit, *J. Chromatogr. Sci.*, 9 (1971) 513.
11 F. J. Evans, *J. Chromatogr.*, 88 (1974) 411.
12 M. C. Castle, *J. Chromatogr.*, 115 (1975) 437.
13 R. E. Majors, *Biochem. Soc. Trans.*, 3 (1975) 867.
14 P. H. Cobb, *Analyst (London)*, 101 (1976) 81.
15 H. Tokunaga, T. Kimura and J. Kawamura, *Iyakuhin Kenkyu*, 7 (1976) 10.
16 W. Linder and R. W. Frei, *J. Chromatogr.*, 117 (1976) 81.
17 F. Nachtmann, H. Spitzy and R. W. Frei, *Anal. Chem.*, 48 (1976) 1576.
18 F. Nachtmann, H. Spitzy and R. W. Frei, *J. Chromatogr.*, 122 (1976) 293.
19 F. Nachtmann, *Z. Anal. Chem.*, 282 (1976) 209.
20 K. Shimada, M. Hasegawa, K. Hasebe, V. Fujii and T. Nambara, *J. Chromatogr.*, 124 (1976) 79.
21 K. Shimada, M. Hasegawa, K. Hasebe, V. Fujii and T. Nambara, *Chem. Pharm. Bull.*, 24 (1976) 2995.
22 F. Erni and R. W. Frei, *J. Chromatogr.*, 130 (1977) 169.
23 J. C. Gfeller, G. Frey and R. W. Frei, *J. Chromatogr.*, 142 (1977) 271.
24 H. Kirchner, J. Jurenitsch and W. Kubelka, *Sci. Pharm.*, 49 (1981) 281.
25 V. Ya. Davydov, A. V. Kiselev, D. M. Popov and Yu. M. Sapojnikov, *Khim.-Farm. Zh.*, 13 (1979) 110.
26 V. Ya. Davydov, A. V. Kiselev and D. M. Popov, *Khim.-Farm. Zh.*, 14 (1980) 111.
27 F. Kaiser, *Chem. Ber.*, 88 (1955) 556.
28 E. Haack, F. Kaiser and H. Spingler, *Naturwissenschaften*, 44 (1957) 633.
29 D. Waldi, *Arch. Pharm. (Weinheim)*, 292 (1959) 206.
30 H. Lichti and A. von Wartburg, *Helv. Chim. Acta*, 43 (1960) 1666.
31 G. Züllich, K. H. Damm and B. P. Lisboa, *J. Chromatogr.*, 115 (1975) 117.
32 L. Nover, G. Baumgarten and M. Luckner, *J. Chromatogr.*, 32 (1968) 93, 123 and 141.
33 L. Nover, G. Jüttner, S. Noack, G. Baumgarten and M. Luckner, *J. Chromatogr.*, 39 (1969) 419 and 450.
34 R. Kaliszan, *J. Chromatogr.*, 220 (1981) 71.
35 E. Tomlinson, *J. Chromatogr.*, 113 (1975) 1.
36 I. F. Makarevich, E. P. Kemertelidze, S. G. Kislichenko, V. V. Zatula, A. A. Reznichenko, D. G. Kolesnikov and I. P. Kovalev, *Kardenolidy i Bufadienolidy (Cardenolides and Bufadienolides)*, Mecniereba, Tbilisi, 1975.
37 A. V. Kiselev, A. A. Atraskova, T. N. Gvozdovitch and Ya. I. Yashin, *J. Chromatogr.*, 195 (1980) 205.
38 V. Ya. Davydov, A. V. Kiselev, D. M. Popov and Yu. M. Sapojnikov, *Khim.-Farm. Zh.*, 12, No. 4 (1978) 102.