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# Lipophilic character of cardiac glycosides

## $R_M$ values as lipophilicity parameters

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SUMMARY

Chromatographic $R_M$ values were measured for a series of 41 cardiac glycosides and aglycones. By means of the $\Delta R_M$ values it was possible to calculate the $R_M$ values for a further 19 compounds. An excellent correlation was found between the present $R_M$ values and those measured or calculated with the Cohnen *et al.* system. In a similar way, the $R_M$ values were shown to be well correlated with both high-performance liquid chromatographic data and octanol–water partition coefficients (log $P$). The additive contribution of each substituent group to the overall lipophilicity of the molecule seems to be constant in each subset of *Digitalis* derivatives.

## INTRODUCTION

The cardiac steroids, despite their long history, are among the most important drugs in modern therapeutics. However, quantitative studies of the relationships between structure and activity (QSAR) of these compounds have received little attention[1–3]. Only in recent times has there been some increasing interest in QSAR studies of cardiac steroids[4–9]. As the lipophilic character is one of the most important physico-chemical factor determining the biological activity of drugs, several workers[4,6,7,10] have studied the determination of lipophilicity indices of cardiac steroids. Nevertheless, there is still a lack of data describing the lipophilic character of most of these compounds. The determination of the classical octanol–water partition coefficient is the main method for establishing the lipophilic character of drugs. However, the $R_M$ and log $k'$ values obtained by reversed-phase partition thin-layer (TLC) and high-performance liquid chromatography (HPLC) were shown to be well

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correlated with the partition coefficients of many chemicals and have been proposed as an alternative method[11,12]. The $R_M$ values were calculated by means of the equation

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

An earlier contribution to the study of the relationship between chemical structure and chromatographic behaviour was provided by Nover and co-workers[13,14] by means of adsorption paper and thin-layer chromatography. With regard to a QSAR study, the purpose of this work was to determine or calculate $R_M$ and $\log k'$ values for a large series of cardiac steroids.

## EXPERIMENTAL

### *Chemicals*

Several cardiac glycosides and aglycones (compounds 4, 8, 41, 43, 44, 45, 46, 55, 57, 58 and 60) were a generous gift from Simes (Milan, Italy). Other compounds were obtained from commercial sources. All the drugs were used without further purification. All solvents were of analytical-reagent or HPLC grade.

### *Determination of $R_M$ values*

The TLC technique had been described previously[15]. Glass plates (20 × 20 cm) were coated with silica gel G (E. Merck, Darmstadt, F.R.G.). In order to control the pH of the stationary phase, a slurry of silica gel G was prepared with 0.09 *M* sodium hydroxide solution. A non-polar stationary phase was obtained by impregnating the silica gel G layer with silicone DC 200 (viscosity 350 cSt) (Applied Science Labs., State College, PA, U.S.A.). The impregnation was carried out by developing the plates in a 5% silicone solution in diethyl ether. Eight plates could be impregnated in a single chromatographic chamber containing 200 ml of the silicone solution. The plates were left in the chamber for 12 h, *i.e.*, for several hours after the silicone solution had reached the top of the plates. The chromatographic chamber was saturated with the vapour of the mobile phase.

A migration distance of 10 cm was obtained on all plates by cutting the layer at 12 cm and spotting the compounds on a line 2 cm from the lower edge of the plate. The mobile phase saturated with silicone oil was aqueous buffer (sodium acetate–Veronal buffer, 1/7 *M* at pH 7.2), alone or mixed with various amounts of acetone. Two plates were developed simultaneously in a chromatographic chamber containing 200 ml of mobile phase, at room temperature.

The cardioactive drugs were dissolved in methanol, acetone or water (1–2 mg/ml) and 1 $\mu$l of solution was spotted randomly on the plates in order to avoid any systematic error. The developed plates were dried and sprayed with an alkaline solution of potassium permanganate. After a few minutes at 120°C, yellow spots appeared on an intense pink background.

### *Determination of log P values*

The *n*-octanol–water partition coefficients of six genins were measured by means of the shake-flask method[16], using distilled water as the polar phase and *n*-octanol as

the lipid phase; each phase was previously saturated with the other one and centrifuged if not clear.

A carefully weighed amount of compound was dissolved in the octanol phase, and an appropriate amount of water was added; the bottles were then shaken gently for *ca.* 5 min and centrifuged for 1.0 h at 2000 rpm (895 *g*). The ratio of the octanol and water volumes was chosen so as to give a reliable UV absorbance at the wavelength of maximum absorption.

The concentration in the aqueous phase was determined spectrophotometrically by means of a Varian DMS-90 UV–visible spectrophotometer; each reported log $P$ value is the average of at least four determinations with $s \leqslant 0.03$.

*Determination of log k' values*

HPLC was performed on a Waters Assoc. 820 chromatography workstation, using a μBondapak $C_{18}$ column (300 × 3.9 mm I.D.) (Waters Assoc., Milford, MA, U.S.A.), packed with silica gel (particle size 10 μm) with a $C_{18}$ chemically bonded non-polar stationary phase[17]. A Waters Assoc. UV detector at 220 nm and Hamilton 802 chromatographic syringes (25 μl) were used. The compounds were dissolved in methanol (1 mg/ml) and applied to the column in 5-μl volumes. All solutions and reagents were first filtered through Millipore filters (Type FH, pore size 0.5 μm). The separation was carried out using acetonitrile–water mixtures as the mobile phase at a flow-rate of 1 ml/min. The acetonitrile concentration ranged from 20–40 to 50–70%. The retention times were expressed as

$$\log k' = \log\left(\frac{t_x - t_0}{t_0}\right)$$

where $t_x$ is the retention time of the compound and $t_0$ that of the solvent front.

## RESULTS

*$R_M$ values and lipophilicity of cardioactive steroids*

The reversed-phase TLC of the cardioactive compounds showed that most of them did not move from the starting line when the mobile phase was aqueous buffer alone. In order to obtain suitable $R_M$ values it was necessary to add acetone to the mobile phase. Only with the six most hydrophilic compounds, ouabain, strophanthidol, k-strophanthidin, k-strophanthin β, k-strophanthoside and digoxigenin, could reliable $R_M$ values be obtained even at 0% acetone in the mobile phase. However, as usual in TLC and HPLC, for all the compounds there was a linear relationship between $R_M$ values and a range of acetone concentration.

The equations describing such a linear relationship allowed the calculation of extrapolated $R_M$ values at 0% acetone in the mobile phase for the compounds that did not migrate with the aqueous buffer alone. The validity of the extrapolation technique is shown by the fact that the experimental $R_M$ values of 0% acetone of the above six most hydrophilic compounds are very close to the extrapolated $R_M$ values, calculated for the same compounds over a wider range of acetone concentrations. All the extrapolated $R_M$ values are reported in Tables I and II.

The range of the linear relationship between $R_M$ values and acetone concen-

TABLE I

$R_M$ VALUES OF CARDIAC GLYCOSIDES AND AGLYCONES OF *DIGITALIS* SPP.

| *Compound* | | *Structure* | | *Empirical formula* | *Molecular weight* | $R_M$[c] | | |
|---|---|---|---|---|---|---|---|---|
| *No.* | *Generic name* | *Genin* | *Side-chain at C-3-β-OH* | | | *This work* | *Cohnen et al.*[10] | *Dzimiri et al.*[7] |
| 1 | Digoxigenin | Digoxigenin | – | $C_{23}H_{34}O_5$ | 390.53 | 0.98 | 0.49 | −0.03 |
| 2 | Digoxigenin monodigitoxoside | Digoxigenin | D-Digitoxose | $C_{29}H_{44}O_8$ | 520.67 | 1.24 | 0.98 | −0.01 |
| 3 | Digoxigenin bisdigitoxoside | Digoxigenin | 2 D-Digitoxose | $C_{35}H_{54}O_{11}$ | 650.81 | 1.65 | 1.23 | 0.17 |
| 4 | Digoxin | Digoxigenin | 3 D-Digitoxose | $C_{41}H_{64}O_{14}$ | 780.92 | 2.01 | 1.78 | 0.23 |
| 5 | α-Acetyldigoxin | Digoxigenin | 3 D-Digitoxose + acetyl | $C_{43}H_{66}O_{15}$ | 822.91 | 2.27 | 2.05 | 0.49 |
| 6 | β-Acetyldigoxin | Digoxigenin | 3 D-Digitoxose + acetyl | $C_{43}H_{66}O_{15}$ | 822.91 | 2.38 | 2.10 | 0.70 |
| 7 | Lanatoside C | Digoxigenin | 3 D-Digitoxose + acetyl + D-glucose | $C_{49}H_{76}O_{20}$ | 985.10 | 2.19 | 1.84 | – |
| 8 | Deslanoside C | Digoxigenin | 3 D-Digitoxose + D-glucose | $C_{47}H_{74}O_{19}$ | 943.11 | 1.86 | 1.55[a] | – |
| 9 | α,β-Diacetyldigoxin | Digoxigenin | 3 D-Digitoxose + 2 acetyl | $C_{45}H_{68}O_{16}$ | 864.95 | 2.89 | 2.37[a] | 1.21 |
| 10 | 12-Acetyldigoxin | 12-Acetyldigoxigenin | 3 D-Digitoxose | $C_{43}H_{66}O_{15}$ | 822.96 | 2.96 | – | 0.89 |
| 11 | 12-Acetyl-β-methyldigoxin | 12-Acetyldigoxigenin | 3 D-Digitoxose + methyl | $C_{44}H_{68}O_{15}$ | 836.99 | 3.45 | – | 1.26 |
| 12 | α-Methyldigoxin | Digoxigenin | 3 D-Digitoxose + methyl | $C_{42}H_{66}O_{14}$ | 795.08 | 2.50 | 2.29[a] | 0.54 |
| 13 | β-Methyldigoxin | Digoxigenin | 3 D-Digitoxose + methyl | $C_{42}H_{66}O_{14}$ | 795.08 | 2.48 | 2.29 | 0.60 |
| 14 | α,β-Dimethyldigoxin | Digoxigenin | 3 D-Digitoxose + 2 methyl | $C_{43}H_{68}O_{14}$ | 809.11 | 2.98[a] | 2.80[a] | 0.94 |
| 15 | Digitoxigenin | Digitoxigenin | – | $C_{23}H_{34}O_4$ | 374.50 | 1.93 | 1.56 | 1.01 |
| 16 | Digitoxigenin monodigitoxoside | Digitoxigenin | D-Digitoxose | $C_{29}H_{44}O_7$ | 504.67 | 2.23 | 2.34 | 1.05 |
| 17 | Digitoxigenin bisdigitoxoside | Digitoxigenin | 2 D-Digitoxose | $C_{35}H_{54}O_{10}$ | 634.81 | 2.65 | 2.44 | 1.28 |
| 18 | Digitoxin | Digitoxigenin | 3 D-Digitoxose | $C_{41}H_{64}O_{13}$ | 764.92 | 3.18 | 2.92 | 1.32 |
| 19 | α-Acetyldigitoxin | Digitoxigenin | 3 D-Digitoxose + acetyl | $C_{43}H_{66}O_{14}$ | 806.91 | 3.54 | 3.21[a] | – |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 20 | β-Acetyldigitoxin | Digitoxigenin | 3 D-Digitoxose + acetyl | $C_{43}H_{66}O_{14}$ | 806.91 | 3.53[a] | 3.21[a] | – |
| 21 | Lanatoside A | Digitoxigenin | 3 D-Digitoxose + acetyl + D-glucose | $C_{49}H_{76}O_{19}$ | 969.10 | 3.38 | 3.09 | – |
| 22 | α,β-Diacetyldigitoxin | Digitoxigenin | 3 D-Digitoxose + 2 acetyl | $C_{45}H_{68}O_{15}$ | 848.95 | 3.88[a] | 3.50[a] | – |
| 23 | β-Methyldigitoxin | Digitoxigenin | 3 D-Digitoxose + methyl | $C_{42}H_{66}O_{13}$ | 778.95 | 3.66[a] | 3.43[a] | 1.71 |
| 24 | Evomonoside[b] | Digitoxigenin | L-Rhamnose | $C_{29}H_{44}O_8$ | 520.67 | 2.28[a] | 1.83 | – |
| 25 | Neriifolin[b] | Digitoxigenin | L-Thevetose | $C_{30}H_{46}O_8$ | 534.70 | 2.14 | 1.72 | – |
| 26 | Gitoxigenin | Gitoxigenin | – | $C_{23}H_{34}O_5$ | 390.50 | 1.72 | 1.37 | 0.38 |
| 27 | Gitoxigenin monodigitoxoside | Gitoxigenin | D-Digitoxose | $C_{29}H_{44}O_8$ | 520.67 | 2.15[a] | 1.86[a] | 0.46 |
| 28 | Gitoxigenin bisdigitoxoside | Gitoxigenin | 2 D-Digitoxose | $C_{35}H_{54}O_{11}$ | 650.81 | 2.58[a] | 2.35[a] | – |
| 29 | Glucogitoroside | Gitoxigenin | D-Digitoxose + D-glucose | $C_{35}H_{54}O_{13}$ | 682.80 | 1.97[a] | 1.63[a] | – |
| 30 | Gitoxin | Gitoxigenin | 3 D-Digitoxose | $C_{41}H_{64}O_{14}$ | 780.92 | 3.00 | 2.83 | 0.68 |
| 31 | α-Acetylgitoxin | Gitoxigenin | 3 D-Digitoxose + acetyl | $C_{43}H_{66}O_{15}$ | 822.91 | 3.35[a] | 3.12[a] | – |
| 32 | β-Acetylgitoxin | Gitoxigenin | 3 D-Digitoxose + acetyl | $C_{43}H_{66}O_{15}$ | 822.91 | 3.35[a] | 3.12[a] | – |
| 33 | Lanatoside B | Gitoxigenin | 3 D-Digitoxose + acetyl + D-glucose | $C_{49}H_{76}O_{20}$ | 985.10 | 3.12 | 2.94[a] | – |
| 34 | α,β-Diacetylgitoxin | Gitoxigenin | 3 D-Digitoxose + 2 acetyl | $C_{45}H_{68}O_{16}$ | 864.95 | 3.70[a] | 3.41[a] | – |
| 35 | Pentaacetylgitoxin | 16-Acetylgitoxigenin | 3 D-Digitoxose + 4 acetyl | $C_{51}H_{74}O_{19}$ | 990.87 | 4.59 | 3.83[a] | 1.86 |
| 36 | 16-Acetylgitoxin | 16-Acetylgitoxigenin | 3 D-Digitoxose | $C_{43}H_{66}O_{15}$ | 822.96 | 2.93 | 2.67[a] | 0.88 |
| 37 | 16-Formylgitoxin | 16-Formylgitoxigenin | 3 D-Digitoxose | $C_{42}H_{64}O_{14}$ | 792.93 | 2.50 | – | 0.84 |
| 38 | Oleandrigenin[b] | 16-Acetylgitoxigenin | – | $C_{25}H_{36}O_6$ | 432.57 | 1.58 | 1.34 | – |
| 39 | Oleandrin[b] | 16-Acetylgitoxigenin | L-Oleandrose | $C_{32}H_{48}O_9$ | 576.70 | 2.34 | 2.26 | – |
| 40 | 16-Desacetyloleandrin[b] | Gitoxigenin | L-Oleandrose | $C_{30}H_{46}O_8$ | 534.66 | 2.44[a] | 2.55 | – |

[a] Calculated $R_M$ values (see text).

[b] Evomonoside, neriifolin, oleandrigenin, oleandrin and 16-desacetyloleandrin are listed in Table I as they are characterized by *Digitalis* aglycones.

[c] TLC system: see text.

TABLE II

$R_M$ VALUES OF CARDIAC GLYCOSIDES AND AGLYCONES FROM OTHER BOTANICAL SOURCES

| *Compound* | | *Structure* | | *Empirical formula* | *Molecular weight* | $R_M$[b] | | |
|---|---|---|---|---|---|---|---|---|
| *No.* | *Generic name* | *Genin* | *Side-chain at C-3-β-OH* | | | *This work* | *Cohnen et al.*[10] | *Dzimiri et al.*[7] |
| 41 | k-Strophanthidin | Strophanthidin | – | $C_{23}H_{32}O_6$ | 404.49 | 0.94 | 0.65 | −0.16 |
| 42 | Strophanthidin-3-acetate | Strophanthidin | Acetyl | $C_{25}H_{34}O_7$ | 446.48 | 1.23 | 0.94[a] | – |
| 43 | Cymarin | Strophanthidin | D-Cymarose | $C_{30}H_{44}O_9$ | 548.65 | 1.34 | 1.23 | −0.10 |
| 44 | k-Strophanthin-β | Strophanthidin | D-Cymarose + D-glucose | $C_{36}H_{54}O_{14}$ | 710.84 | 1.02 | 1.00[a] | −0.50 |
| 45 | k-Strophanthoside | Strophanthidin | D-Cymarose + 2 D-glucose | $C_{42}H_{64}O_{13}$ | 873.03 | 0.99 | 0.77[a] | −0.62 |
| 46 | Convallatoxin | Strophanthidin | L-Rhamnose | $C_{29}H_{42}O_{10}$ | 550.63 | 1.29 | 1.26[a] | – |
| 47 | Desglucocheirotoxin | Strophanthidin | D-Gulomethylose | $C_{29}H_{42}O_{10}$ | 550.63 | 1.29[a] | 0.92[a] | – |
| 48 | Olitoriside | Strophanthidin | D-Boivinose + D-glucose | $C_{35}H_{52}O_{14}$ | 696.79 | 1.10[a] | 0.91[a] | – |
| 49 | Helveticoside | Strophanthidin | D-Digitoxose | $C_{29}H_{42}O_9$ | 534.63 | 1.21 | 1.14[a] | – |
| 50 | Erysimoside | Strophanthidin | D-Digitoxose + D-glucose | $C_{35}H_{52}O_{14}$ | 696.79 | 1.03[a] | 0.91[a] | – |
| 51 | Strophanthidol | Strophanthidol | – | $C_{23}H_{34}O_6$ | 406.50 | 0.73 | 0.46[a] | – |
| 52 | Cymarol | Strophanthidol | D-Cymarose | $C_{30}H_{46}O_9$ | 550.65 | 1.19 | 1.04 | – |
| 53 | Convallatoxol | Strophanthidol | L-Rhamnose | $C_{29}H_{44}O_{10}$ | 552.65 | 1.11[a] | 1.07 | – |
| 54 | Helveticosol | Strophanthidol | D-Digitoxose | $C_{29}H_{44}O_9$ | 536.65 | 1.03[a] | 0.95[a] | – |
| 55 | Heptaacetyl-k-strophanthoside | Strophanthidin | D-Cymarose + 2 D-glucose + 7 acetyl | $C_{56}H_{78}O_{26}$ | 1166.9 | 3.41 | 2.80[a] | – |
| 56 | Ouabagenin | Ouabagenin | – | $C_{23}H_{24}O_8$ | 438.50 | 0.18[a] | −0.05[a] | – |
| 57 | Ouabain | Ouabagenin | L-Rhamnose | $C_{29}H_{44}O_{12}$ | 584.64 | 0.53 | 0.22 | −0.70 |
| 58 | Proscillaridin | Scillarenin | L-Rhamnose | $C_{30}H_{42}O_8$ | 530.64 | 2.15 | 1.92 | – |
| 59 | Scillaren A | Scillarenin | L-Rhamnose + D-glucose | $C_{36}H_{52}O_{13}$ | 692.78 | 1.97[a] | 1.69[a] | – |
| 60 | Peruvoside | Cannogenin | L-Thevetose | $C_{30}H_{44}O_9$ | 548.65 | 1.68 | – | – |

[a] Calculated $R_M$ values (see text).
[b] TLC system: see text.

trations is limited by the fact that at lower and higher acetone concentrations all the compounds tend not to move from the starting line or to migrate with the solvent front, respectively, *i.e.*, to deviate from the linear relationship. Therefore, the extrapolated $R_M$ values in Tables I and II were obtained from equations calculated by means of $R_M$

TABLE III

RANGES OF ACETONE CONCENTRATIONS USED FOR THE CALCULATION OF THE TLC EQUATIONS

| *Compound No.* | *Acetone range (%)* | *TLC equation* | | |
|---|---|---|---|---|
| | | $a = R_M$ | $b$ | $r$ |
| 57 | 0–24 | 0.535 ± 0.019 | −0.066 ± 0.001 | 0.996 |
| 51 | 0–24 | 0.732 ± 0.071 | −0.033 ± 0.002 | 0.985 |
| 41 | 0–24 | 0.938 ± 0.098 | −0.038 ± 0.038 | 0.967 |
| 1 | 0–32 | 0.980 ± 0.047 | −0.048 ± 0.002 | 0.992 |
| 45 | 0–32 | 0.991 ± 0.162 | −0.058 ± 0.007 | 0.961 |
| 44 | 0–36 | 1.022 ± 0.184 | −0.063 ± 0.011 | 0.959 |
| 52 | 4–32 | 1.194 ± 0.043 | −0.050 ± 0.002 | 0.995 |
| 49 | 4–36 | 1.213 ± 0.080 | −0.045 ± 0.003 | 0.982 |
| 42 | 4–40 | 1.230 ± 0.100 | −0.043 ± 0.003 | 0.987 |
| 2 | 4–24 | 1.240 ± 0.118 | −0.063 ± 0.007 | 0.972 |
| 46 | 4–32 | 1.295 ± 0.090 | −0.059 ± 0.004 | 0.983 |
| 43 | 4–24 | 1.343 ± 0.085 | −0.065 ± 0.005 | 0.986 |
| 38 | 8–36 | 1.581 ± 0.070 | −0.043 ± 0.002 | 0.991 |
| 3 | 8–32 | 1.650 ± 0.112 | −0.063 ± 0.005 | 0.983 |
| 60 | 8–50 | 1.684 ± 0.117 | −0.050 ± 0.004 | 0.976 |
| 26 | 8–50 | 1.719 ± 0.044 | −0.050 ± 0.001 | 0.992 |
| 8 | 8–40 | 1.859 ± 0.131 | −0.068 ± 0.005 | 0.981 |
| 15 | 16–50 | 1.932 ± 0.058 | −0.050 ± 0.002 | 0.996 |
| 4 | 16–50 | 2.010 ± 0.094 | −0.062 ± 0.003 | 0.989 |
| 25 | 16–50 | 2.141 ± 0.221 | −0.055 ± 0.006 | 0.986 |
| 58 | 16–50 | 2.149 ± 0.120 | −0.054 ± 0.003 | 0.985 |
| 7 | 16–50 | 2.187 ± 0.120 | −0.066 ± 0.003 | 0.990 |
| 16 | 20–50 | 2.232 ± 0.122 | −0.058 ± 0.004 | 0.990 |
| 5 | 20–50 | 2.268 ± 0.118 | −0.063 ± 0.003 | 0.994 |
| 39 | 20–50 | 2.345 ± 0.139 | −0.057 ± 0.004 | 0.989 |
| 6 | 24–50 | 2.377 ± 0.119 | −0.067 ± 0.003 | 0.994 |
| 13 | 20–50 | 2.477 ± 0.057 | −0.070 ± 0.002 | 0.985 |
| 12 | 20–50 | 2.503 ± 0.095 | −0.071 ± 0.003 | 0.995 |
| 37 | 20–60 | 2.504 ± 0.124 | −0.062 ± 0.003 | 0.991 |
| 17 | 28–55 | 2.653 ± 0.147 | −0.065 ± 0.003 | 0.993 |
| 9 | 28–55 | 2.887 ± 0.122 | −0.070 ± 0.003 | 0.994 |
| 36 | 28–50 | 2.933 ± 0.285 | −0.068 ± 0.009 | 0.976 |
| 10 | 28–55 | 2.962 ± 0.209 | −0.072 ± 0.005 | 0.988 |
| 30 | 32–40 | 3.000 ± 0.016 | −0.076 ± 0.001 | 0.999 |
| 33 | 28–50 | 3.121 ± 0.113 | −0.078 ± 0.003 | 0.996 |
| 18 | 28–60 | 3.183 ± 0.141 | −0.070 ± 0.003 | 0.992 |
| 21 | 28–55 | 3.380 ± 0.120 | −0.072 ± 0.003 | 0.996 |
| 55 | 28–50 | 3.406 ± 0.120 | −0.066 ± 0.003 | 0.996 |
| 11 | 28–50 | 3.446 ± 0.303 | −0.081 ± 0.008 | 0.985 |
| 19 | 36–50 | 3.540 ± 0.450 | −0.074 ± 0.010 | 0.981 |
| 35 | 40–60 | 4.588 ± 0.800 | −0.088 ± 0.017 | 0.966 |

values determined with acetone concentrations ranging from 0 to 24% or from 36–40 to 50–60% depending on the lipophilicity of the test compounds. The most hydrophilic compound, ouabain, shows a linear relationship between $R_M$ values and acetone concentration in the range 0–24%. For the most lipophilic compound, penta-acetylgitoxin, acetone concentrations ranging from 40 to 60% were used.

The ranges of acetone concentrations and the TLC equations are reported in Table III, where $a$ and $b$ are the intercept and slope, with their standard errors, respectively, and $r$ is the correlation coefficient. The intercepts ($a = R_M$) are also reported in Tables I and II. In Table III the compounds are listed in order of increasing lipophilicity to show the good correlation between extrapolated $R_M$ values and ranges of acetone concentrations. The slopes in Table III show that the equations describe a series of almost parallel straight lines.

Cohnen *et al.*[10] measured the $R_M$ values of a series of cardioactive steroids by means of a TLC technique which seems to be very similar to our own system. Their extrapolated $R_M$ values at 0% acetone in the mobile phase are reported in Tables I and II. A very good correlation is shown by eqn. 1 between the present $R_M$ values and those obtained by Cohnen *et al.* [10] for a series of 23 compounds for which the experimental $R_M$ values were available in both TLC systems.

$$R_M = 0.361\ (\pm\ 0.063) + 0.937\ (\pm\ 0.033)\ R_{M\text{Cohnen}} \quad (1)$$
$$(n = 23;\ r = 0.987;\ s = 0.121;\ F = 784.7;\ P < 0.005)$$

In eqn. 1 and all subsequent equations, $n$ is number of data points, $r$ is the correlation coefficient, $s$ is the standard error of the equation and $F$ is the value of the $F$-test.

The experimental $R_M$ values provide some understanding of the influence of substituent groups determining the lipophilicity of the whole molecule. The *Digitalis* glycosides can be grouped into three families on the basis of the aglycones (Table I). Cardiac glycosides and aglycones from different botanical sources are listed in Table II. The presence of digitoxosyl, acetyl or methyl group(s) in the sugar residue at C-3 increases the lipophilic character. The $\Delta R_M$ values reported in Table IV were used in the calculation of the $R_M$ values of the mono- and bisdigitoxosides and also acetyl derivatives of trisdigitoxosides for which the experimental $R_M$ values were not available. In the Cohnen *et al.* system the $R_M$ value of helveticoside was obtained by adding the average $\Delta R_M$ value of the digitoxosyl group to the $R_M$ value of strophanthidin.

The $R_M$ values of desacetyllanatoside C, strophanthidin-3-acetate, pentaacetyl-gitoxin and heptaacetyl-k-strophanthoside in the Cohnen *et al.* system were calculated from the experimental $R_M$ values for lanatoside C, strophanthidin, 16-acetylgitoxin and k-strophanthoside by subtracting or adding an average $\Delta R_M$ of 0.29 for each of the acetyl groups.

$$R_M\text{ (lanatoside B)} = R_M\text{ (gitoxin)} + [R_M\text{ (lanatoside A)} - R_M\text{ (digitoxin)}]$$
$$= 2.83 + (3.09 - 2.92) = 3.00$$
$$R_M\text{ (lanatoside B)} = R_M\text{ (gitoxin)} + [R_M\text{ (lanatoside C)} - R_M\text{ (digoxin)}]$$
$$= 2.83 + (1.84 - 1.78) = 2.89$$
$$\bar{x} = 2.94$$

TABLE IV

INFLUENCE OF SUBSTITUENT GROUPS ON THE LIPOPHILIC CHARACTER

| *Group* | *Position* | *Compounds* | $\Delta R_M$ | $\Delta R_M$ *(Cohnen et al.*[10]*)* |
|---|---|---|---|---|
| OH | C-16 | Gitoxin *vs.* digitoxin | −0.18 | −0.09 |
| | | Gitoxigenin *vs.* digitoxigenin | −0.21 | −0.19 |
| | | | $\bar{x}=-0.19$ | $\bar{x}=-0.14$ |
| | C-12 | Digoxin *vs.* digitoxin | −1.17 | −1.14 |
| | | Digoxigenin monodigitoxoside *vs.* digitoxigenin monodigitoxoside | −0.99 | −1.36 |
| | | Digoxigenin bisdigitoxoside *vs.* digitoxigenin bisdigitoxoside | −1.00 | −1.21 |
| | | Digoxigenin *vs.* digitoxigenin | −0.95 | −1.07 |
| | | | $\bar{x}=-1.03$ | $\bar{x}=-1.19$ |
| $OCOCH_3$ | C-16 | Oleandrigenin *vs.* digitoxigenin | −0.35 | −0.22 |
| | | 16-Acetylgitoxin *vs.* digitoxin | −0.25 | – |
| | | | $\bar{x}=-0.30$ | |
| | C-12 | 12-Acetyldigoxin *vs.* digitoxin | −0.22 | – |
| $OCOCH_3$ *vs.* OH | C-16 | Oleandrin *vs.* 16-desacetyloleandrin | – | −0.29 |
| | | Oleandrigenin *vs.* gitoxigenin | −0.14 | −0.03 |
| | | 16-Acetylgitoxin *vs.* gitoxin | −0.07 | – |
| | | | $\bar{x}=-0.10$ | $\bar{x}=-0.16$ |
| | C-12 | 12-Acetyldigoxin *vs.* digoxin | 0.95 | – |
| | | 12-Acetyl-β-methyldigoxin *vs.* β-methyldigoxin | 0.97 | – |
| | | | $\bar{x}=0.96$ | |
| $CH_2OH$ *vs.* CHO | C-10 | Cymarol *vs.* cymarin | −0.15 | −0.19 |
| | | Strophanthidol *vs.* strophanthidin | −0.21 | – |
| | | | $\bar{x}=-0.18$ | |
| CHO *vs.* OH | C-16 | 16-Formylgitoxin *vs.* gitoxin | −0.50 | – |
| $OCOCH_3$ *vs.* OH | Side chain | α-Acetyldigitoxin *vs.* digitoxin | 0.36 | – |
| | | α-Acetyldigoxin *vs.* digoxin | 0.26 | 0.27 |
| | | β-Acetyldigoxin *vs.* digoxin | 0.37 | 0.32 |
| | | α,β-Diacetyldigoxin *vs.* digoxin/2 | 0.44 | – |
| | | Pentaacetylgitoxin *vs.* 16-acetylgitoxin/4 | 0.41 | – |
| | | Heptaacetyl-k-strophanthoside *vs.* k-strophanthoside/7 | 0.34 | – |
| | | | $\bar{x}=0.35$ | $\bar{x}=0.29$ |
| Glucosyl *vs.* OH | Side-chain | Deslanoside *vs.* digoxin | −0.15 | – |
| | | Lanatoside C *vs.* α-acetyldigoxin | −0.08 | −0.21 |
| | | Lanatoside C *vs.* β-acetyldigoxin | −0.19 | −0.26 |
| | | Lanatoside A *vs.* α-acetyldigitoxin | −0.16 | – |
| | | k-Strophanthin β *vs.* cymarin | −0.32 | – |
| | | k-Strophanthoside *vs.* cymarin/2 | −0.17 | – |
| | | | $\bar{x}=-0.18$ | $\bar{x}=-0.23$ |

*(Continued on p. 172)*

TABLE IV *(continued)*

| *Group* | *Position* | *Compounds* | $\Delta R_M$ | $\Delta R_M$ *(Cohnen et al.*[10]*)* |
|---|---|---|---|---|
| Rhamnosyl *vs.* OH | Side-chain | Convallatoxin *vs.* k-strophanthidin | 0.35 | – |
| | | Evomonoside *vs.* digitoxigenin | – | 0.27 |
| Digitoxosyl *vs.* OH | Side-chain | Digoxigenin monodigitoxoside *vs.* digoxigenin | 0.26 | 0.49 |
| | | Digoxigenin bisdigitoxoside *vs.* digoxigenin/2 | 0.33 | 0.37 |
| | | Digoxin *vs.* digoxigenin/3 | 0.34 | 0.43 |
| | | Digitoxigenin monodigitoxoside *vs.* digitoxigenin | 0.30 | 0.78 |
| | | Digitoxigenin bisdigitoxoside *vs.* digitoxigenin/2 | 0.36 | 0.44 |
| | | Digitoxin *vs.* digitoxigenin/3 | 0.42 | 0.45 |
| | | Gitoxin *vs.* gitoxigenin/3 | 0.43 | 0.49 |
| | | Helveticoside *vs.* k-strophanthidin | 0.27 | – |
| | | 16-Acetylgitoxin *vs.* oleandrigenin/3 | 0.45 | – |
| | | | $\bar{x}=$ 0.35 | $\bar{x}=$ 0.49 |
| Cymarosyl *vs.* OH | Side-chain | Cymarin *vs.* k-strophanthidin | 0.40 | 0.58 |
| | | Cymarol *vs.* strophanthidol | 0.46 | – |
| | | | $\bar{x}=$ 0.43 | |
| Oleandrosyl *vs.* OH | Side-chain | Oleandrin *vs.* oleandrigenin | 0.76 | 0.92 |
| Thevetosyl *vs.* OH | Side-chain | Neriifolin *vs.* digitoxigenin | 0.21 | 0.16 |
| $OCH_3$ *vs.* OH | Side-chain | α-Methyldigoxin *vs.* digoxin | 0.49 | – |
| | | β-Methyldigoxin *vs.* digoxin | 0.47 | 0.51 |
| | | 12-Acetyl-β-methyldigoxin *vs.* 12-acetyldigoxin | 0.49 | – |
| | | | $\bar{x}=$ 0.48 | |

The $\Delta R_M$ value of the glucosyl group in the sugar residue was used for the calculation of the $R_M$ values of glucogitoroside, erysimoside, scillaren A, k-strophanthin β and k-strophanthoside by adding it for one or two residues to the $R_M$ values of gitoxigenin monodigitoxoside, helveticoside, proscillaridin and cymarin. The $R_M$ values of evomonoside and ouabagenin were calculated from the $R_M$ values of digitoxigenin and ouabain by adding or subtracting, respectively, the $R_M$ value of the rhamnosyl group in the sugar residue.

The $\Delta R_M$ value of the acetoxyl group *vs.* OH at C-16 was used in the calculation of the $R_M$ values of 16-acetylgitoxin and desacetyloleandrin by adding or subtracting it, respectively, from the $R_M$ values of gitoxin and oleandrin.

The $R_M$ values of helveticosol, convallatoxol, strophanthidol and convallatoxin were calculated by adding or subtracting the $\Delta R_M$ value for $CH_2OH$ *vs.* CHO at C-10 from the $R_M$ values of helveticoside, convallatoxin, strophanthidin and convallatoxol.

The $R_M$ values of desglucocheirotoxin and olitoriside were calculated in both systems by adding the $\Delta R_M$ values of gulomethylose, or boivinose and glucose, respectively. As gulomethylose and boivinose are the isomeric forms of rhamnose and digitoxose, respectively, the $\Delta R_M$ values of the latter forms were used (Table IV). In fact, Davydov[6] had calculated the same retention values for both pairs of isomers. However, the results for another pair of isomers must be pointed out, *i.e.*, cymarose and oleandrose, whereas Davydov[6] had calculated the same retention values for these sugar residues, in the present and the Cohnen *et al.* system the $\Delta R_M$ values are different.

Finally, the $R_M$ values of α-methyldigoxin, β-methyldigitoxin and α,β-dimethyldigoxin were calculated by means of the $\Delta R_M$ values for the α- and/or β-methyl groups in the side-chain.

The equation

$$R_M = 0.202\ (\pm 0.046) + 1.027\ (\pm 0.021)\ R_{M\ \text{Cohnen}} \quad (2)$$
$$(n = 56;\ r = 0.988;\ s = 0.153;\ F = 2265.3;\ P < 0.005)$$

calculated by means of both experimental and calculated $R_M$ values is very similar to eqn. 1 and shows that the calculated $R_M$ values do not deviate from the relationship described by eqn. 1 for the experimental $R_M$ values. The slopes in both eqns. 1 and 2 are very close to unity, which explains why the $\Delta R_M$ values in Table IV are fairly close in the two systems.

Hence any substituent group tends to induce the same variation of lipophilic character in both TLC systems. Intercepts higher than zero indicate a systematic difference between the two systems, probably due to the different kind of silicone oil used by Cohnen *et al.*[10]. Eqn. 2 holds over a wide range of $R_M$ values, with a difference on a logarithmic scale of 4.65, which means a 44 668-fold difference in lipophilicity.

More recently, Dzimiri *et al.*[7] measured the $R_M$ values of a series of cardiotonic steroids by means of a different TLC system, which had also been used by Cohnen *et al.*[10]. The stationary phase was characterized by the presence of octanol instead of silicone oil. The mobile phase was methanol–water (30:70), which yielded the experimental $R_M$ values reported in Tables I and II. When a compound had been tested also by Cohnen *et al.*[10], an average $R_M$ value is reported in Tables I and II.

The following equation describes the relationship between our $R_M$ values and those measured by Dzimiri *et al.*[7]:

$$R_M = 1.477\ (\pm 0.092) + 1.331\ (\pm 0.105)\ R_{M\ \text{Dzimiri}} \quad (3)$$
$$(n = 28;\ r = 0.927;\ s = 0.365;\ F = 159.0;\ P < 0.005)$$

The correlation coefficient is not as good as that for eqns. 1 and 2. A better equation was obtained when compounds 1, 15, 16, 30 and 35, which showed the largest deviations, were excluded from the analysis:

$$R_M = 1.532\ (\pm 0.064) + 1.286\ (\pm 0.078)\ R_{M\ \text{Dzimiri}} \quad (4)$$
$$(n = 23;\ r = 0.963;\ s = 0.240;\ F = 269.8;\ P < 0.005)$$

A justification for excluding those compounds might be that they were the most deviant also when correlating the present $R_M$ values with Dzimiri *et al.*'s log $P$ values

TABLE V

OCTANOL–WATER PARTITION COEFFICIENTS AND HPLC RETENTION DATA FOR CARDIAC GLYCOSIDES

| *Compound No.* | *Log P* | *Log P (Dzimiri et al.[7])* | *Log k'* | *ln V (Davydov et al.[4,6])* | *Log k' (Dzimiri et al.[7])* | *Compound No.* | *Log P* | *Log P (Dzimiri et al.[7])* | *Log k'* | *ln V (Davydov et al.[4,6])* | *Log k' (Dzimiri et al.[7])* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.14 | 1.12 | 1.23 | – | −0.44 | 31 | – | – | – | – | – |
| 2 | – | 1.09 | 1.29 | – | −0.43 | 32 | – | – | – | – | – |
| 3 | – | 1.23 | 1.40 | – | −0.36 | 33 | – | – | 2.01 | 3.94 | – |
| 4 | – | 1.26 | 1.59 | 3.36 | −0.30 | 34 | – | – | – | – | – |
| 5 | – | 1.72 | 1.93 | – | −0.01 | 35 | – | 3.46 | 2.75 | – | 1.69 |
| 6 | – | 1.98 | 1.95 | – | 0.10 | 36 | – | 2.15 | 1.95 | – | 0.36 |
| 7 | – | – | 1.60 | 3.25 | – | 37 | – | 2.04 | 2.10 | – | 0.26 |
| 8 | – | – | 1.72 | 2.75 | – | 38 | 2.09 | – | 1.63 | – | – |
| 9 | – | 2.52 | 1.87 | – | 0.63 | 39 | – | – | 1.85 | 4.01 | – |
| 10 | – | 2.29 | 2.25 | – | 0.20 | 40 | – | – | – | – | – |
| 11 | – | 2.87 | 2.29 | – | 0.61 | 41 | 0.64 | 0.61 | 1.12 | 2.18 | −0.38 |
| 12 | – | 1.75 | 1.92 | – | 0.05 | 42 | – | – | 1.33 | 2.54 | – |
| 13 | – | 1.76 | 1.98 | – | 0.08 | 43 | – | 0.64 | 1.49 | 2.90 | −0.10 |
| 14 | – | 2.14 | 2.28 | – | 0.44 | 44 | – | −0.96 | 1.32 | 2.07 | −0.66 |
| 15 | 2.64 | 2.41 | 1.70 | – | 0.26 | 45 | – | −0.82 | 1.15 | 1.69 | −0.68 |
| 16 | – | 2.49 | 1.79 | – | 0.28 | 46 | – | – | 1.65 | 1.91 | – |
| 17 | – | 2.45 | 1.74 | – | 0.38 | 47 | – | – | – | 2.11 | – |
| 18 | – | 2.83 | 2.04 | 4.89 | 0.52 | 48 | – | – | – | 1.95 | – |
| 19 | – | – | 2.37 | 5.47 | – | 49 | – | – | 1.57 | 2.45 | – |
| 20 | – | – | – | – | – | 50 | – | – | – | 1.86 | – |
| 21 | – | – | 2.30 | 4.78 | – | 51 | – | – | 1.12 | – | – |
| 22 | – | – | – | – | – | 52 | – | – | 1.13 | – | – |
| 23 | – | 3.11 | – | – | 0.92 | 53 | – | – | – | – | – |
| 24 | – | – | – | – | – | 54 | – | – | – | – | – |
| 25 | – | – | 1.72 | – | – | 55 | – | – | 2.07 | – | – |
| 26 | 1.76 | 1.64 | 1.46 | – | −0.17 | 56 | −0.02 | – | 0.83 | – | – |
| 27 | – | 1.78 | – | – | −0.08 | 57 | – | −1.85 | 0.75 | 0.62 | −0.85 |
| 28 | – | – | – | – | – | 58 | – | – | 1.52 | – | – |
| 29 | – | – | – | 2.33 | – | 59 | – | – | 1.74 | – | – |
| 30 | – | 1.67 | 1.94 | 4.11 | 0.11 | 60 | – | – | 1.48 | – | |

(eqn. 5). In the correlation with the log $k'$ values (see eqn. 8) they were not excluded, but again they were among the most deviant compounds.

The higher intercepts in eqns. 3 and 4 are due to the fact that Dzimiri *et al.*[7] used a stationary phase containing octanol instead of silicone oil. The higher slopes are due to the narrower range of Dzimiri *et al*'s $R_M$ values. In eqns. 3 and 4 about 7–14% of the variance in our $R_M$ values is not explained by the regression.

*Relationship between $R_M$ and log P or HPLC data*

The log $P$ and log $k'$ values of the cardiac glycosides are reported in Table V, where most of the data available in the literature are also listed. Cohnen *et al.*[10] and Dzimiri *et al.*[7] measured the octanol–water partition coefficients of cardiac steroids. The best correlation between $R_M$ and log $P$ values was found by Dzimiri *et al.*[7]. Therefore, in Table V we report only the log $P$ values of the compounds for which an $R_M$ value in Dzimiri *et al.*'s system was available. For the compounds tested by both Cohnen *et al.*[10] and Dzimiri *et al.*[7] an average log $P$ is reported in Table V. Eqn. 5, excluding compounds 1, 15, 16, 30 and 35, and eqn. 6, considering only the six genins, can be compared with eqn. 7, calculated with Dzimiri *et al.*'s $R_M$ and log $P$ values:

$$R_M = 1.248\ (\pm 0.118) + 0.634\ (\pm 0.061) \log P_{\text{Dzimiri}} \quad (5)$$
$$(n=23;\ r=0.914;\ s=0.362;\ F=107.0;\ P<0.005)$$

$$R_M = 0.345\ (\pm 0.145) + 0.637\ (\pm 0.088) \log P \quad (6)$$
$$(n=6;\ r=0.964;\ s=0.193;\ F=52.2;\ P<0.005)$$

$$R_{M\ \text{Dzimiri}} = -0.252\ (\pm 0.068) + 0.516\ (\pm 0.034) \log P_{\text{Dzimiri}} \quad (7)$$
$$(n=28;\ r=0.948;\ s=0.215;\ F=233.5;\ P<0.005)$$

The different intercepts in eqns. 5 and 6 compared with eqn. 7 are due to the use of octanol instead of silicone oil in the Dzimiri *et al.* TLC stationary phase. The difference between the intercepts in eqns. 5 and 6 is due simply to the high standard error in eqn. 5 and to the fact that the two equations share only two compounds (nos. 26 and 41). On the other hand, the slopes of the three equations are very close.

Obviously the results with eqns. 5 and 7 could have been expected on the basis of eqn. 4. The log $k'$ values reported in Table V were extrapolated to 0% acetonitrile in the mobile phase from the linear relationship between log $k'$ and acetonitrile concentration as already described[17]. In Table V the HPLC data measured by Davydov[6] and Dzimiri *et al.*[7] are also listed. The relationship between the $R_M$ values and the HPLC retention data is described by the following equations:

$$R_M = -1.488\ (\pm 0.192) + 2.109\ (\pm 0.110) \log k' \quad (8)$$
$$(n=44;\ r=0.947;\ s=0.306;\ F=369.5;\ P<0.005)$$

$$R_M = -0.249\ (\pm 0.170) + 0.716\ (\pm 0.054) \ln V \quad (9)$$
$$(n=21;\ r=0.950;\ s=0.297;\ F=176.1;\ P<0.005)$$

$$R_M = 2.109\ (\pm 0.064) + 1.680\ (\pm 0.121) \log k'_{\text{Dzimiri}} \quad (10)$$
$$(n=28;\ r=0.938;\ s=0.336;\ F=192.0;\ P<0.005)$$

The ln $V$ term in eqn. 9 is the retention index used by Davydov[6].

In eqns. 5 and 6 and 8–10, again about 7–17% of the variance in the $R_M$ values is not explained by the regression. Nevertheless, the log $P$ values and the HPLC data from two and three different laboratories, respectively, seem to agree with the $R_M$ values as lipophilic indices of the cardiac steroids.

## DISCUSSION

The $\Delta R_M$ values in Table IV can be used in order to describe the contribution of substituent groups to the lipophilicity of the whole molecule. As regards the Digitalis genins, the lipophilic character decreases in the order digitoxigenin > gitoxigenin > digoxigenin. In fact, attaching an OH group at C-12 (digoxigenin) or C-16 (gitoxigenin) decreases the lipophilic character of the parent compound, digitoxigenin. According to Dzimiri *et al.*[7], digoxigenin is more hydrophilic than gitoxigenin as the OH group at C-12 is more exposed to the complementary hydroxyl groups of the aqueous phase than the OH group at C-16. A similar conclusion can be drawn by considering the fragment values for the hydroxyl group at C-12 and C-16. In fact, the $\Delta$log $P$ values for the pairs digoxigenin–digitoxigenin and gitoxigenin–digitoxigenin are $-1.50$ and $-0.88$, respectively (Table V). Therefore, the $\Delta$log $P$ value for the OH at C-12 is much closer to the aliphatic fragment value of $-1.64$ for the OH group[18] than that for the OH at C-16. On the other hand, the acetyloxy group has the same hydrophilic character at both C-12 and C-16. Apparently the lactone ring is not able to mask the acetyloxy group at C-16. As a consequence, acetylation of the OH group has opposite effects at C-16 and C-12. k-Strophanthidin with an OH group at C-5 and a formyl group at C-10 is slightly more hydrophilic than digoxigenin. Ouabagenin (g-strophanthidin) is the most hydrophilic genin because of the addition of two other OH groups at C-1 and C-11 and the replacement of the formyl group with a more hydrophilic $CH_2OH$ group at C-10. On the other hand, the replacement of the OH group at C-16 with a formyl group makes 16-formylgitoxin less lipophilic than gitoxin.

The sugar residues at C-3 examined in the present and Cohnen *et al.*'s system, *i.e.*, oleandrose, cymarose, digitoxose, rhamnose, thevetose and glucose, have polarities increasing in that order. As mentioned above, Davydov[6] obtained the same order of ranking except for oleandrose and cymarose, for which the same retention value was obtained. The introduction of an $\alpha$- and/or $\beta$-methyl group and an acetyl group(s) into the sugar side-chain increases the lipophilicity. It may be noted that our experimental $R_M$ values for heptaacetyl-k-strophanthoside and pentaacetylgitoxin, 3.41 and 4.59, respectively, are very close to those calculated in the present system from the $R_M$ value of k-strophantoside and 16-acetylgitoxin, 3.44 and 4.33, respectively. The use of the average $\Delta R_M$ for the acetyl group in the side-chain in the calculation of the $R_M$ value for strophantidin-3-acetate in the Cohnen *et al.* system may also be pointed out. This seems to be justified by the fact that in the present system the $OCOCH_3$ *vs.* OH group at C-3 has a $\Delta R_M$ value of 0.29 which is not far from the average $\Delta R_M$ of 0.35 reported in Table IV for any acetyl group in the side-chain.

The additivity of the lipophilic contribution of any substituent group at C-3 seems to rule out any significant kind of interaction between the steroid nucleus and the sugar side-chain. Fig. 1 shows histograms which illustrate the increments in lipophilicity due to the addition of the same sugar residue in each family of genins. It is shown that in each of the *Digitalis* derivatives families the lipophilicity increases in the

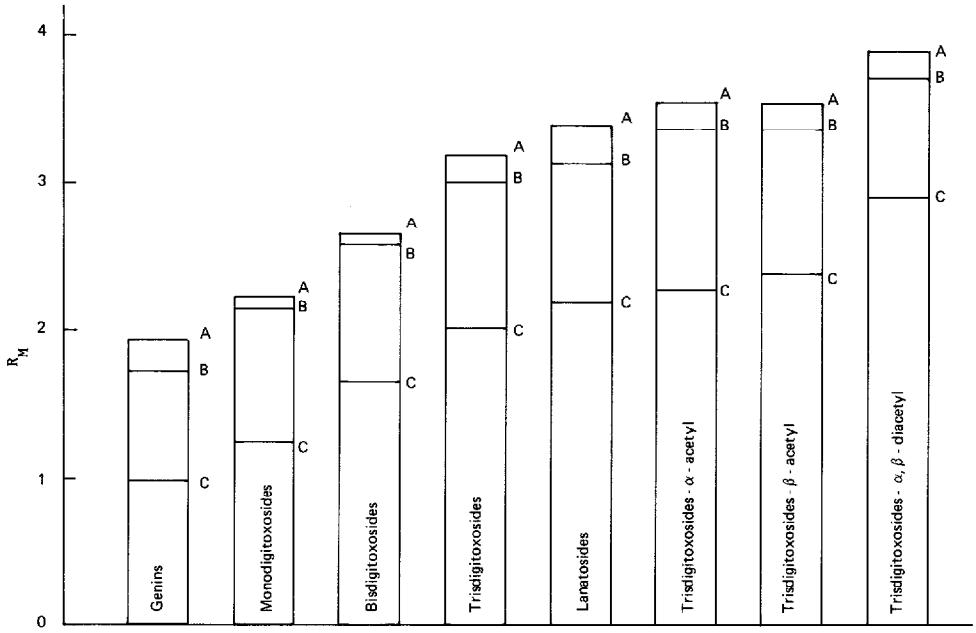


Fig. 1. Influence of side-chain composition on the overall lipophilicity of *Digitalis* cardiac glycosides. The different subsets are (A) digitoxigenin, (B) gitoxigenin and (C) digoxigenin.

order genin < monodigitoxoside < bisdigitoxside < trisdigitoxoside < α- or β-acetyl < α,β-diacetyl. Lanatoside A, B and C are less lipophilic than the α- or β-acetyl derivatives because of the presence of a glucosyl group in the side-chain.

A comment is deserved from a more general point of view. The present $R_M$ values were obtained by extrapolation from the linear relationship between $R_M$ values and acetone concentrations in the mobile phase. The extrapolation technique allows the calculation of a theoretical $R_M$ value at 0% acetone in the mobile phase, *i.e.*, in a standard system where all the compounds could be compared.

Another great advantage over the determination of the $R_M$ values at only one organic solvent concentration in the mobile phase is that in this way one can avoid the error that might arise because of different slopes of the straight lines describing the relationship between $R_M$ values and organic solvent concentration in the mobile phase. Two compounds might have the same $R_M$ value at a given organic solvent concentration and different extrapolated $R_M$ values. Finally, the extrapolation procedure tends to yield a wider range of $R_M$ values, which is reflected in the slopes of eqns. 3 and 4. In fact, the $R_M$ values in the octanol system were measured at only one organic solvent concentration in the mobile phase.

## CONCLUSION

The chromatographic data and the partition coefficients from the literature agree well with the present $R_M$ values in describing the lipophilicity of cardiac steroids. The reliability of the $\Delta R_M$ values in Table IV is an important aspect. In fact, the

fragment values can be used in calculating the $R_M$ values of other cardiac steroids. Finally, this work seems to have demonstrated some advantages of reversed-phase TLC or any other chromatographic system over the use of the classical partition coefficient[7,19]. The chromatographic method is simple and rapid; it requires little material, which is important with compounds that are expensive and/or difficult to synthesize, such as the cardiac steroids; the material does not need to be very pure; and the detection of spots by non-specific methods avoids the need for specific quantitative analytical methods.

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