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LIQUID ION EXCHANGERS IN PAPER CHROMATOGRAPHY OF STEROIDAL GLUCOSIDURONIC ESTERS

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SUMMARY

Twenty-five steroidal glucosiduronic esters were chromatographed on paper in each of five straight-phase systems and five reversed-phase systems. The resolving characteristics of these systems were compared by expressing the retention values in terms of R_M and correlating the data by use of linear regression equations. The resolving characteristics of the straight-phase systems as a group are very similar, and those of the reversed-phase systems are somewhat less similar; the various systems differ considerably in polarity. The resolving properties of a straight-phase system and a reversed-phase system which contain the same liquid ion exchanger are markedly different, a circumstance which increases the probability that a pair of compounds which was not separated in a straight-phase system will be resolved in the reversed-phase system which contains the same liquid ion exchanger. The migration of the esters in the foregoing systems was compared with the migration of the corresponding glucosiduronic acids (a previous study) in the same solvent systems. If a particular pair of conjugates is not separable as carboxylic acids in a specific straight-phase system, there is a relatively good chance that they will be separable as methyl esters in the same system.

INTRODUCTION

In previous studies we chromatographed a collection of steroidal glucosiduronic acids in a group of straight-phase[1–3] and reversed-phase[4] solvent systems and compared the resolving properties of the various systems. More recently, we have considered whether a mixture of these steroidal glucosiduronates would be more readily resolved in the form of methyl esters than as the carboxylic acids. Consequently, we chromatographed the methyl esters in each of five straight-phase systems and five reversed-phase systems and compared the extent of resolution achieved on a mixture of conjugates in the form of free acids with that achieved in the form of methyl esters. This paper gives a resumé of our findings. The resolving properties of the various solvent systems are correlated by use of linear regression equations.

MATERIALS AND METHODS

Sources of ion exchangers, glucosiduronic esters, and other chemicals have been given previously[1–3]. Chromatographic procedures which were described earlier for straight-phase[1–3] and for reversed-phase[4] systems were followed. The steroidal glucosiduronic esters which were chromatographed are listed in Table I. Straight-phase systems, which consisted predominantly of chloroform and formamide, were modified by making the mobile phase of 0.10 *N* with an ion exchanger*; no counterion was added to the formamide phase. Reversed-phase systems employed 0.20 *M* aqueous KCl as mobile phase on sheets of paper which had been dipped in a 0.050 *M* solution of exchanger** in chloroform and allowed to dry in air for about 5 min before the compounds to be chromatographed were applied.

TABLE I

LIST OF STEROIDAL GLUCOSIDURONIC ESTERS

The numbers used for the esters correspond to those assigned to the acids in previous papers[1–4].

Ester	*Name*
1	Methyl (3,20-dioxopregn-4-en-21-yl β-D-glucopyranosid)uronate
2	Methyl (17-hydroxy-3,20-dioxopregn-4-en-21-yl β-D-glucopyranosid)uronate
3	Methyl (21-hydroxy-20-oxo-5β-pregnan-3α-yl β-D-glucopyranosid)uronate
4	Methyl (3α-hydroxy-20-oxo-5β-pregnan-21-yl β-D-glucopyranosid)uronate
5	Methyl (3,11,20-trioxopregn-4-en-21-yl β-D-glucopyranosid)uronate
6	Methyl (11β-hydroxy-3,20-dioxopregn-4-en-21-yl β-D-glucopyranosid)uronate
7	Methyl (17-hydroxy-3,11,20-trioxopregn-4-en-21-yl β-D-glucopyranosid)uronate
8	Methyl (3α-17-dihydroxy-20-oxo-5β-pregnan-21-yl β-D-glucopyranosid)uronate
9	Methyl [(18R)(11β,18-epoxy-21-hydroxy-3,20-dioxopregn-4-en-18-yl α-D-glucopyranosid)]-uronate
10	Methyl (3α-hydroxy-11,20-dioxo-5β-pregnan-21-yl β-D-glucopyranosid)uronate
11	Methyl (21-hydroxy-11,20-dioxo-5β-pregnan-3α-yl β-D-glucopyranosid)uronate
12	Methyl (11β,17-dihydroxy-3,20-dioxopregn-4-en-21-yl β-D-glucopyranosid)uronate
13	Methyl (17,21-dihydroxy-20-oxo-5β-pregnan-3α-yl β-D-glucopyranosid)uronate
14	Methyl (3α,11β-dihydroxy-20-oxo-5β-pregnan-21-yl β-D-glucopyranosid)uronate
15	Methyl (3α,17-dihydroxy-11,20-dioxo-5β-pregnan-21-yl β-D-glucopyranosid)uronate
16	Methyl (17,21-dihydroxy-11,20-dioxo-5β-pregnan-3α-yl β-D-glucopyranosid)uronate
17	Methyl (20β,21-dihydroxy-11-oxo-5β-pregnan-3β-yl β-D-glucopyranosid)uronate
18	Methyl (11β,21-dihydroxy-20-oxo-5β-pregnan-3α-yl β-D-glucopyranosid)uronate
20	Methyl (3α,11β,17-trihydroxy-20-oxo-5β-pregnan-21-yl β-D-glucopyranosid)uronate
21	Methyl (17,20β,21-trihydroxy-11-oxo-5β-pregnan-3α-yl β-D-glucopyranosid)uronate
22	Methyl (11β,17,21-trihydroxy-20-oxo-5β-pregnan-3α-yl β-D-glucopyranosid)uronate
24	Methyl (11β,17,20β,21-tetrahydroxy-5β-pregnan-3α-yl β-D-glucopyranosid)uronate
26	Dimethyl (20-oxo-5β-pregnan-3α,21-ylene di-β-D-glucopyranosid)uronate
27	Dimethyl (11,20-dioxo-5β-pregnan-3α-21-ylene di-β-D-glucopyranosid)uronate
28	Dimethyl (11β-hydroxy-20-oxo-5β-pregnan-3α,21-ylene di-β-D-glucopyranosid)uronate

* Straight-phase systems are designated by the exchanger employed and the subscript SP. Exchangers used in straight-phase systems were: tetraheptylammonium acetate (TA·OAc); tetraheptylammonium chloride (TA·Cl); tetraheptylammonium sulfate [$(TA)_2 \cdot SO_4$]; tri-*n*-octylamine hydrochloride (TOA·HCl); and tri-*n*-octylamine hydrosulfate [$(TOA \cdot H)_2SO_4$].

** Reversed-phase systems are designated by the exchanger employed and the subscript RP. Exchangers used for reversed-phase systems were: Amberlite LA-2 hydrochloride (ALA-2·HCl); Amberlite XLA-3 hydrochloride (XLA-3·HCl); methyltricaprylylammonium chloride (Aliquat); TA·Cl; and TOA·HCl.

RESULTS AND DISCUSSION

R_F values were determined for 22 monoglucosiduronic esters and three diglucosiduronic esters in five straight-phase systems (Table II) and in five reversed-phase

TABLE II

R_F AND R_M VALUES OF STEROIDAL GLUCOSIDURONIC ESTERS IN STRAIGHT-PHASE SYSTEMS

The mobile phase was 0.10 *N* ion exchanger in chloroform; stationary phase was formamide. Glucosiduronic esters are listed in Table I.

Ester	*System*									
	TOA·HCl$_{SP}$		*(TOA·H)*$_2$*SO*$_{4SP}$		*TA·Cl*$_{SP}$		*TA·OAc*$_{SP}$		*(TA)*$_2$*·SO*$_{4SP}$	
	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
1	0.38	0.21	0.63	−0.23	0.77	−0.53	0.84	—	—	—
2	0.067	1.14	0.23	0.53	0.32	0.33	0.68	−0.33	0.74	−0.45
3	0.33	0.31	0.57	−0.12	0.72	−0.41	0.81	—	—	—
4	0.14	0.79	0.35	0.27	0.49	0.02	0.73	−0.43	—	—
5	0.13	0.83	0.34	0.29	0.45	0.09	0.74	−0.45	—	—
6	0.055	1.24	0.20	0.60	0.30	0.37	0.69	−0.35	—	—
7	0.026	1.57	0.11	0.91	0.18	0.66	0.55	−0.09	0.64	−0.25
8	0.020	1.69	0.069	1.13	0.12	0.87	0.44	0.10	0.57	−0.12
9	0.090	1.00	0.33	0.31	0.35	0.27	0.71	−0.39	—	—
10	0.032	1.48	0.11	0.91	0.19	0.63	0.53	−0.05	0.64	−0.25
11	0.072	1.11	0.23	0.53	0.28	0.41	0.63	−0.23	0.69	−0.35
12	0.010	—	0.038	1.40	0.068	1.14	0.33	0.31	0.54	−0.07
13	0.034	1.45	0.14	0.79	0.21	0.58	0.55	−0.09	0.67	−0.31
14	0.014	—	0.053	1.25	0.080	1.06	0.36	0.25	0.51	−0.02
15	—	—	0.026	1.57	0.052	1.26	0.27	0.43	0.36	0.25
16	0.011	—	0.046	1.32	0.086	1.03	0.39	0.19	0.53	−0.05
17	—	—	0.053	1.25	0.10	0.95	0.45	0.09	0.53	−0.05
18	0.026	1.57	0.090	1.00	0.12	0.87	0.43	0.12	0.52	−0.04
20	—	—	—	—	0.016	1.79	0.12	0.87	0.25	0.48
21	—	—	0.018	1.74	0.030	1.51	0.26	0.45	0.41	0.16
22	—	—	0.013	—	0.021	1.67	0.13	0.83	0.26	0.45
24	—	—	—	—	—	—	0.10	0.95	0.27	0.43
26	—	—	0.044	1.34	0.059	1.20	0.51	−0.02	0.65	−0.27
27	—	—	0.014	—	0.026	1.57	0.31	0.35	0.54	−0.07
28	—	—	—	—	—	—	0.14	0.79	0.36	0.25

systems (Table III). In these tables mobility of the compounds is expressed also in terms[5] of R_M; $R_M = \log [(1/R_F) - 1]$. The properties of two chromatography systems relative to a series of compounds can be compared by plotting values for migration in one system against those in another system. If mobility is expressed as R_F, a curvilinear relationship is usually obtained; when mobility is expressed as R_M, a linear relationship is commonly observed. Rather than plotting the R_M values on a graph, we have elected to use the R_M values in linear regression equations for comparing the chromatographic characteristics of the various solvent systems with those of one solvent system which is taken as a standard. The equations, which are analogous to those

TABLE III

R_F AND R_M VALUES OF STEROIDAL GLUCOSIDURONIC ESTERS IN REVERSED-PHASE SYSTEMS

The mobile phase was 0.20 *M* aqueous KCl; the paper was impregnated with 0.050 *M* exchanger in chloroform. Glucosiduronic esters are listed in Table I.

Ester	System									
	TA·Cl$_{RP}$		*Aliquat*$_{RP}$		*TOA·HCl*$_{RP}$		*ALA-2·HCl*$_{RP}$		*XLA-3·HCl*$_{RP}$	
	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
1	0.028	1.54	0.048	1.30	0.20	0.60	0.48	0.04	0.45	0.09
2	0.019	1.71	0.057	1.22	0.18	0.66	0.50	0.00	0.58	−0.14
3	—	—	0.013	—	0.027	1.56	0.12	0.87	0.16	0.72
4	—	—	0.011	—	0.024	1.61	0.12	0.87	0.12	0.87
5	0.15	0.75	0.25	0.48	0.55	−0.09	0.72	−0.41	0.69	−0.35
6	0.048	1.30	0.078	1.07	0.31	0.35	0.61	−0.19	0.59	−0.16
7	0.077	1.08	0.23	0.53	0.50	0.00	0.72	−0.41	0.73	−0.43
8	—	—	0.014	—	0.032	1.48	0.18	0.66	0.23	0.53
9	0.26	0.45	0.32	0.33	0.63	−0.23	0.75	−0.48	0.71	−0.39
10	0.029	1.53	0.049	1.29	0.22	0.55	0.49	0.02	0.43	0.12
11	0.041	1.37	0.061	1.19	0.27	0.43	0.56	−0.10	0.50	0.00
12	0.035	1.44	0.10	0.95	0.30	0.37	0.64	−0.25	0.67	−0.31
13	—	—	0.014	—	0.030	1.51	0.16	0.72	0.26	0.45
14	0.019	1.71	0.040	1.38	0.16	0.72	0.49	0.02	0.46	0.07
15	0.024	1.61	0.075	1.09	0.21	0.58	0.59	−0.16	0.59	−0.16
16	0.028	1.54	0.070	1.12	0.23	0.53	0.59	−0.16	0.61	−0.19
17	0.031	1.50	0.057	1.22	0.25	0.48	0.59	−0.16	0.49	0.02
18	0.018	1.74	0.047	1.31	0.16	0.72	0.52	−0.04	0.53	−0.05
20	0.024	1.61	0.069	1.13	0.18	0.66	0.58	−0.14	0.58	−0.14
21	0.058	1.21	0.13	0.83	0.44	0.10	0.72	−0.41	0.66	−0.29
22	0.027	1.56	0.081	1.06	0.23	0.53	0.62	−0.21	0.64	−0.25
24	0.072	1.11	0.15	0.75	0.46	0.07	0.74	−0.45	0.69	−0.35
26	—	—	0.018	1.74	0.12	0.87	0.49	0.02	0.27	0.43
27	0.074	1.10	0.11	0.91	0.54	−0.07	0.76	−0.50	0.58	−0.14
28	0.040	1.38	0.088	1.02	0.45	0.09	0.75	−0.48	0.58	−0.14

discussed in detail previously[5], are of the following general form where symbols *a* and *b* represent slope and intercept in the equation for a straight line.

$$R_M(\text{system } Y) = a \cdot R_M(\text{system } X) + b \qquad (1)$$

Of primary importance in the interpretation of regression equations is the coefficient of correlation, *r*, which may range from 1.00 (perfect correlation) to 0.00 (no correlation) to −1.00 (perfect inverse correlation). In the present application, the commonly used coefficient *r* is a curvilinear measure of the degree of similarity in the chromatographic migration of a group of compounds in two solvent systems (*X* and *Y*). The expression $\sqrt{1-r^2}$ is a linear measure of the degree of similarity in the resolving properties of two systems, and thus it is simpler to interpret. When applied to chromatographic data for a group of compounds in two systems, $\sqrt{1-r^2}$ gives the fractional amount of the resolving power[6] of system *Y*, in terms of standard deviation of mean

R_M, which is unique and useful for separating a mixture of components not separated by system X. When applied to chromatographic data from a single system on two related groups of compounds (*e.g.*, parent compounds and homogeneous derivatives), $\sqrt{1-r^2}$ gives the fractional amount of the resolving power of the system which is unique for the derivatives and is useful for separating as derivatives mixtures that were not separated as parent compounds*.

Fig. 1 shows the relationship between values for r and $\sqrt{1-r^2}$. It is apparent that as values for r decrease from 1.00 to slightly lower amounts, corresponding values for $\sqrt{1-r^2}$ increase very rapidly. Thus, when $r = 0.97$, $\sqrt{1-r^2} \approx 0.25$, and when $r = 0.85$, $\sqrt{1-r^2} \approx 0.50$. A value of 0.50 for $\sqrt{1-r^2}$ implies that, in terms of R_M, 50% of the intrinsic resolving power of system Y is available for resolving a mixture of compounds not separated by system X.

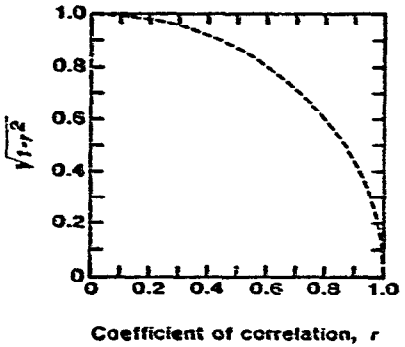

Fig. 1. Relationship between r and $\sqrt{1-r^2}$ for application to linear regression analysis.

An additional attribute which may be useful in describing eqn. 1 is a/r; this value expresses the ratio of the standard deviation of the mean R_M in system Y to the standard deviation of the mean R_M in system X. If the value for a/r is larger than 1.00, system Y offers the greater probability in terms of R_M of separating two randomly selected substances; if the value is less than 1.00, system X is superior.

The data on the monoglucosiduronic esters (compounds 1–24) in Table II have been evaluated by designating the TA·Cl_{SP} systems as system X in eqn. 1 and deriving regression equations for the four other systems relative to this standard. The resulting equations are summarized in Table IV. In each case, r is greater than 0.97, a value which indicates that the resolving properties of the five systems are very similar (see Fig. 1). Values for a/r indicate that the TOA·HCl_{SP} and $(TOA\cdot H)_2SO_{4SP}$ systems give slightly better resolution of the esters than the standard system and that the TA·OAc_{SP} and $(TA)_2\cdot SO_{4SP}$ systems give poorer resolution. Examination of the values for R_F in Table II, or values for b in Table IV, show that the systems differ markedly in polarity, TOA·HCl_{SP} being least polar and $(TA)_2\cdot SO_{4SP}$ being most polar.

* Simultaneously, $\sqrt{1-r^2}$ gives the fractional amount of resolving power which is unique for parent compounds and is useful for separating as parent compounds mixtures which were not separated as derivatives.

TABLE IV

COMPARISON OF STRAIGHT-PHASE SYSTEMS FOR CHROMATOGRAPHY OF STEROIDAL MONOGLUCOSIDURONIC ESTERS

Values in the table apply to the equation $R_M(\text{system } Y) = a \cdot R_M(\text{TA}\cdot\text{C}_{18}) + b$; r is the coefficient of correlation; n is the number of compounds considered in the correlation.

System Y	*a*	*b*	*r*	*n*
$\text{TOA}\cdot\text{HCl}_{SP}$	1.07	0.76	0.990	13
$(\text{TOA}\cdot\text{H})_2\text{SO}_{4SP}$	1.01	0.22	0.992	19
$\text{TA}\cdot\text{OAc}_{SP}$	0.76	−0.56	0.987	19
$(\text{TA})_2\cdot\text{SO}_{4SP}$	0.61	−0.64	0.971	15

For correlation of the data on the reversed-phase systems (Table III), $\text{TA}\cdot\text{Cl}_{RP}$ was used as system X in eqn. 1; the resulting regression equations are summarized in Table V. Values for r indicate that systems designated $\text{ALA-2}\cdot\text{HCl}_{2P}$ and $\text{XLA-3}\cdot\text{HCl}_{RP}$ have resolving properties for the esters that are considerably different from those of the standard system. The best resolution of the esters by a reversed-phase

TABLE V

COMPARISON OF REVERSED-PHASE SYSTEMS FOR CHROMATOGRAPHY OF STEROIDAL GLUCOSIDURONIC ESTERS

Values in the table apply to the equation $R_M(\text{system } Y) = a \cdot R_M(\text{TA}\cdot\text{Cl}_{RP}) + b$; r is the coefficient of correlation; n is the number of compounds considered in the correlation.

System Y	*a*	*b*	*r*	*n*
Aliquat_{RP}	0.83	−0.13	0.927	18
$\text{TOA}\cdot\text{HCl}_{RP}$	0.82	−0.74	0.965	18
$\text{ALA-2}\cdot\text{HCl}_{RP}$	0.42	−0.77	0.835	18
$\text{XLA-3}\cdot\text{HCl}_{RP}$	0.34	−0.63	0.673	18

system, as indicated by values for a/r, is achieved by use of $\text{TA}\cdot\text{Cl}_{RP}$. The general order of decreasing polarity (*i.e.*, increasing R_F for reversed-phase systems) is $\text{TA}\cdot\text{Cl}_{RP} > \text{Aliquat}_{RP} > \text{TOA}\cdot\text{HCl}_{RP} > \text{ALA-2}\cdot\text{HCl}_{RP} \approx \text{XLA-3}\cdot\text{HCl}_{RP}$.

$$R_M(\text{TOA}\cdot\text{HCl}_{RP}) = -0.095\, R_M(\text{TOA}\cdot\text{HCl}_{SP}) + 0.81;\ n = 13;\ r = -0.069 \quad (2)$$

$$R_M(\text{TA}\cdot\text{Cl}_{RP}) = 0.20\, R_M(\text{TA}\cdot\text{Cl}_{SP}) + 1.23;\ n = 17;\ r = 0.349 \quad (3)$$

Comparison of the mobility of the esters in a straight-phase system with their mobility in a reversed-phase system contains the same exchanger is shown in eqns. 2 and 3. It is apparent from the values for r that there is very little correlation between the migration of the esters in corresponding straight-phase and reversed-phase systems. This observation is supported by comparison of data[7] from the corresponding pairs of systems which employ Aliquat, ALA-2·HCl and XLA-3·HCl in which $r =$ 0.054, −0.45 and −0.42, respectively.

Linear regression equations of the following general form were used also to compare the chromatographic migration of the monoglucosiduronic esters with that

of the corresponding monoglucosiduronic acids in the same solvent system or in comparable* systems.

$$R_M(\text{Ester}) = a \cdot R_M(\text{Acid}) + b \quad (4)$$

The equations are summarized in Table VI. For all of the straight-phase systems and the Aliquat_{RP} system, values of r indicate a moderate correlation between migration of the glucosiduronates as esters and as acids ($0.74 < r < 0.89$). Interpretation of these values of r in terms of $\sqrt{1 - r^2}$ (see Fig. 1) implies that if an arbitrarily chosen pair of conjugates was not separated in a particular solvent system as glucosiduronic acids that about half of the intrinsic resolving power of the system would be available to separate the pair as glucosiduronic esters.

TABLE VI

COMPARISON OF THE MIGRATION OF GLUCOSIDURONIC ACIDS AND ESTERS IN COMPARABLE SYSTEMS

Values are from the equation $R_M(\text{ester}) = a \cdot R_M(\text{acid}) + b$. Data are from Tables II and III and refs. 1 and 4.

System	*a*	*b*	*r*	*a/r*	*n*
A. Straight-phase					
$\text{TOA}\cdot\text{HCl}_{SP}$	0.81	0.57	0.854	0.95	13
$(\text{TOA}\cdot\text{H})_2\text{SO}_{4SP}$	0.84	0.57	0.869	0.97	18
$\text{TA}\cdot\text{Cl}_{SP}$	1.03	−0.03	0.884	1.17	21
$\text{TA}\cdot\text{OAc}_{SP}$	0.85	0.10	0.859	0.99	19
$(\text{TA})_2\cdot\text{SO}_{4SP}$	0.86	0.30	0.749	1.15	13
B. Reversed-phase					
$\text{TA}\cdot\text{Cl}_{RP}$	0.88	0.51	0.967	0.91	18
Aliquat_{RP}	0.68	0.35	0.859	0.79	18
$\text{TOA}\cdot\text{HCl}_{RP}$	0.92	−0.15	0.973	0.95	22
$\text{ALA-2}\cdot\text{HCl}_{RP}$	0.79	−0.30	0.981	0.81	22
$\text{XLA-3}\cdot\text{HCl}_{RP}$	0.65	−0.54	0.954	0.68	22

In the straight-phase systems (Table VI), values for a/r indicate that the conjugates are resolved as well in the form of esters as in the form of acids. However, in the reversed-phase systems, the conjugates are resolved better as acids.

It has been postulated[8–10] that if there were no interaction among the various structural components of a molecule, the change in R_M (ΔR_M) associated with a structural alteration at a single location would be constant. Under such circumstances, ΔR_M of esterification for a group of glucosiduronic acids in a specific solvent system should be a constant. The equation relating migration of the esters to that of the acids in a specific system would be $R_M(\text{ester}) = 1.00\, R_M(\text{acid}) + \Delta R_M$ and the coefficient of correlation would be 1.00.

* The straight-phase systems used for the acids[1] consisted of 0.10 *N* exchanger in chloroform and 0.10 *N* counterion in formamide. Since addition of counterion to the stationary phase does not affect the migration of the esters significantly[3], the counterion was omitted from the systems used with the esters.

A summary of ΔR_M values obtained from chromatography of the glucosiduronic acids and esters in four solvent systems is given in Table VII. When all of the conjugates are included in one group (Table VII, line 1), there is a large average deviation from the mean ΔR_M value with three of the systems and a small deviation with the other one. If the glucosiduronates are grouped according to various structural similarities of the compounds, the average deviations of the mean ΔR_M values are reduced with each of the four systems; the values are reduced markedly with the three systems which initially had large average deviations. This observation is illustrated graphically for the TA·Cl_{SP} system in Fig. 2. In the regression equation for the line in the left panel (all conjugates in one group), the value of r is 0.884; values of r for lines in the right panel (conjugates grouped by structural similarities) are greater than 0.990. Analogous relationships between constancy of ΔR_M and structure are observed when the data from the other straight-phase systems, or from the $Aliquat_{SP}$ system are plotted in a similar manner (plots not shown).

TABLE VII

MEAN AND AVERAGE DEVIATION OF ΔR_M VALUES FOR CONVERSION OF GLUCOSIDURONIC ACID TO GLUCOSIDURONIC METHYL ESTER

Data are from Tables II and II and from refs. 1 and 4.

Types of compounds compared	*System*			
	TA·Cl_{SP}	*$(TOA{\cdot}H)_2SO_{4SP}$*	*$Aliquat_{SP}$*	*TA·Cl_{RP}*
All monoglucosiduronates	0.01 ± 0.25	−0.52 ± 0.23	−0.04 ± 0.18	−0.40 ± 0.08
17-Hydroxy compounds conjugated at C-21	−0.37 ± 0.03	−0.84 ± 0.04	0.15 ± 0.03	−0.32 ± 0.03
11β-Hydroxy-17-deoxy compounds conjugated at C-21	−0.17 ± 0.03	−0.74 ± 0.02	0.08 ± 0.01	−0.30 ± 0.03
17-Deoxy compounds conjugated at C-21 with either 11-deoxy or 11-oxo group	0.14 ± 0.04	−0.51 ± 0.01	0.05 ± 0.06	−0.34 ± 0.02
17-Hydroxy compounds conjugated at C-3	0.10 ± 0.06	−0.28 ± 0.03	−0.23 ± 0.06	−0.47 ± 0.07
17-Deoxy compounds conjugated at C-3	0.36 ± 0.09	−0.25 ± 0.03	−0.19 ± 0.05	−0.49 ± 0.05

By using the data in ref. 1 and in Table II, values were calculated for the ΔR_M of hydroxylation at C-17 in the glucosiduronic acids and esters in the TA·Cl_{SP} system. The mean values for ΔR_M of hydroxylation at C-17 in six pairs of C-21 conjugates and in four pairs of C-3 conjugates are given below.

ΔR_M (17α-hydroxylation) for esters conjugated at C-21 = 0.74
ΔR_M (17α-hydroxylation) for acids conjugated at C-21 = 0.32
Difference = 0.42

ΔR_M (17α-hydroxylation) for esters conjugated at C-3 = 0.74
ΔR_M (17α-hydroxylation) for acids conjugated at C-3 = 0.49
Difference = 0.25

These values indicate that in the esters a 17α-hydroxyl group is more polar (retards migration more) than in the acids. In addition, the values also indicate that while position of conjugation has no appreciable effect on polarity of the 17α-hydroxyl group in the esters, the function is less polar in acids conjugated at C-21 than in acids conjugated at C-3 .The foregoing comparisons provide an example of strong interactions among the various structural elements within a compound being chromato-

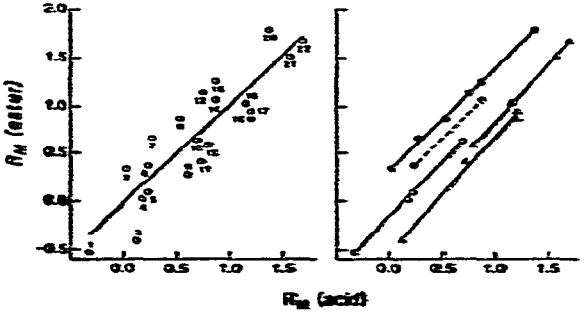

Fig. 2. Comparison of the chromatographic migration (R_M scale) of steroidal glucosiduronic esters and glucosiduronic acids in comparable straight-phase systems containing TA-CI; data are from Table II and ref. 1. In the left panel, the conjugates are compared as a group; the equation of the line is R_M(ester) = 1.03 R_M(acid) − 0.02. In the right panel, the conjugates are divided according to the following structural similarities: ●, 17-hydroxy compounds conjugated at C-21; ◑, 11β-hydroxy-17-deoxy compounds conjugated at C-21; ○, 17-deoxy compounds conjugated at C-21 with either 11-deoxy or 11-oxo group; ▲, 17-hydroxy compounds conjugated at C-3; △, 17-deoxy compounds conjugated at C-3. The equations of the lines are: ●, R_M(ester) = 1.07 R_M(acid) + 0.33; ○, R_M(ester) + 1.14 R_M(acid) − 0.16; ▲, R_M(ester) + 1.20 R_M(acid) − 0.36; △, R_M(ester) = 1.21 R_M(acid) − 0.53.

graphed and/or of large differences in the extent to which some of the structural elements interact with components of the solvent system. The variability of the interactions in glucosiduronic acids which are conjugated at different positions, as opposed to findings in the esters, leads to a variability in ΔR_M values and makes accurate prediction of R_M values for the compounds impossible. However, these same variations of interaction make it possible, in some cases, to separate a pair of substances after they have been derivatized when it was not possible to separate them as the parent compounds.

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