LIQUID ION EXCHANGERS IN PAPER CHROMATOGRAPHY OF STEROIDAL GLUCOSIDURONIC ACIDS

INFLUENCE OF DIFFERENT EXCHANGERS ON THE MOBILITY IN CHLOROFORM–FORMAMIDE AND CORRELATION OF CHROMATOGRAPHIC DATA

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(Received January 13th, 1975)

SUMMARY

A group of 25 steroidal glucosiduronic acids was chromatographed on paper in chloroform–formamide in the presence of several different liquid ion exchangers. Chromatograms were run also in three Bush-type systems. R_F values were converted into R_M values and the data were correlated by use of a series of regression equations of the type $R_M(Y) = a \cdot R_M(X) + b$, in which X designates a standard system to which each other system (Y) is compared. The ratio of the slope a to the correlation coefficient r (*i.e.*, a/r) is a measure of the resolving power of system Y relative to the standard system; intercept b, in association with slope a, is an indication of the polarity of system Y relative to X. The correlation coefficient r and the standard error of estimate $sy \cdot x$ are indications of whether solvent systems Y and X have very similar or relatively different resolving properties for a group of solutes. The regression equations are useful for correlating chromatographic data obtained from a group of compounds in several solvent systems.

Properties of the chromatography systems are discussed and the relative importance of ion exchange and hydrogen bonding with the various solvent systems is pointed out. ΔR_{M_g} and ΔR_{M_r} values are given for functional groups at several locations in the conjugates for ten of the chromatography systems.

INTRODUCTION

Anionic liquid ion exchangers increase the solubility[1] of steroidal glucosiduronic acids in certain nonpolar solvents. As a consequence, relatively polar glucosiduronic acids can be chromatographed in comparatively nonpolar solvent systems if an appropriate ion exchanger is present in the less polar phase.

An initial paper[1] reported that when a particular liquid ion exchanger (tetraheptylammonium chloride) was used in association with a group of chromatography systems (chloroform–formamide, toluene–ethylene glycol, etc.) the acids moved at

different rates relative to one another in the various solvents. A subsequent report[2] discussed the effect of changes in the concentration of liquid ion exchanger and of counterion on the mobility of a group of steroidal glucosiduronic acids.

The present paper is an attempt to correlate chromatographic data which were obtained on a group of steroidal glucosiduronic acids (1) employing various liquid ion exchangers* in chloroform–formamide, and (2) employing several conventional Bush-type solvent systems.

The properties of two chromatographic systems relative to a series of compounds can be compared by plotting mobilities (R_M scale) of individual compounds in one solvent system against those in another. Plots of this kind have indicated[3-5] linear relationships which can be expressed mathematically by use of regression equations. Such equations were used initially by Collander[6] to express the relationship between the partition coefficients of a series of solutes in two solvent systems; subsequent investigators have correlated a considerable amount of partition data by use of regression equations and Leo et al.[7] have summarized these results in an extensive review.

In this paper we correlate our chromatographic data by use of the following equation:

$$R_M(Y) = a \cdot R_M(X) + b$$

R_M designates[8] $\log[(1/R_F) - 1]$ for each compound in the group of substances being compared: R_M has an inverse relationship to R_F and the R_M value of a compound increases with its affinity for the stationary phase. Y represents chromatography system Y, and X designates chromatography system X which is taken as a standard. Symbols a and b represent slope and intercept. Symbols n, r, and $sy \cdot x$, which are used in subsequent paragraphs, represent the number of compounds in the series, the correlation coefficient, and the standard error of estimate**, respectively.

The ratio a/r represents the extent, in terms of R_M, to which a group of compounds is spread out in system Y relative to system X during chromatography; it is equivalent to the ratio of the standard deviation of the R_M values from the mean in system Y to the standard deviation of the R_M values from the mean R_M in system X. Thus, $a/r = S[R_M(Y)]/S[R_M(X)]$ where S represents standard deviation. If a/r is greater than 1.0, the compounds will be spread out more in system Y; if less, they will be spread out more in system X. If the correlation coefficient $r \approx 1.0$, the slope a is a measure of the extent to which the compounds are separated in systems Y and X.

Intercept b is the value of R_M for system Y when R_M in system $X = 0.00$. If $a \approx 1.00$, and correlation coefficient $r \approx 1.00$, b is a measure of the polarity or hydrophilicity of solvent system Y relative to system X. Under the foregoing conditions, if b is positive, compounds will migrate less in system Y than in system X, and vice versa.

Correlation coefficient r expresses the goodness with which R_M values in system Y are predicted from corresponding R_M values in system X. When $r \approx 1.00$,

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* The organic amines and amine salts employed in this paper are referred to as "liquid ion exchangers" even though these substances may participate in the interchange of neutral compounds between the phases of a chromatography system.

** Also $sy \cdot x$ is referred to as standard deviation of residuals and standard deviation from regression[9-11].

the equation predicts the R_M values of compounds in system Y very faithfully: when $r = 0.0$, there is no correlation between the R_M values found experimentally in system Y and those predicted from R_M values in system X. If r is between 0.00 and -1.00, a negative correlation exists: This relationship might be observed when comparing a "straight phase" and a "reversed-phase" system. As r decreases progressively from 1.00 toward 0.00, systems X and Y become successively less alike and the probability that two compounds that migrate at essentially the same rate in system X will migrate at significantly different rates in system Y increases accordingly. Furthermore, if the equation relating system X to a third system (system Z) gives a value for r which is significantly different from the value for r in the equation relating system Y to system X, then system Y and system Z also possess different discriminating properties.

The standard error of estimate ($sy \cdot x$) measures the standard deviation of the differences between the values found for R_M in system Y and those calculated for R_M in system Y. It is an indication, in R_M units, of the difference in properties of systems Y and X. As the value for $sy \cdot x$ decreases and approaches 0.00, resolving properties of systems Y and X become more alike and approach equality; as the value for $sy \cdot x$ increases, the resolving probability of system Y for pairs of compounds not resolved in system X increases.

Regression equations of the foregoing type, when employed with their accompanying qualifiers[9-11] (a or a/r, b, r, and $sy \cdot x$), provide a very useful shorthand procedure for interrelating the chromatographic properties of a group of solvent systems for a series of compounds.

MATERIALS AND METHODS

Sources of chemicals used and chromatographic techniques employed in association with the liquid ion exchangers have been described previously[1,2].

The hydrosulfates of TOA[2], ALA-2[2], and XLA-3[2] were prepared by washing a 0.1 N chloroform solution of the free amine two times with twice its volume of a 0.1 N solution of sulfuric acid. The organic phase was concentrated *in vacuo* to approximately one-third its volume, diluted with chloroform, titrated with sodium dodecyl sulfate, and adjusted to the proper volume. Paper liners in jars for Bush-type systems were wetted with 500 ml of stationary phase and 500 ml of mobile phase were added directly to the bottom of the jar. Freshly prepared jars were equilibrated for at least 20 h before being used and were renewed after use for one week. Chromatography papers which contained conjugates were equilibrated for 2 h in the butanol–water system and for 1 h in the 1,2-dichloroethane–*tert.*-butanol–water–acetic acid system and the butyl acetate–*n*-butanol–water–acetic acid system. Running time in the butanol–water system was about 15 h, that for the latter two systems about 5.5 h.

If the chromatography papers contain a liquid ion exchanger, all compounds in this communication, except the 20,21-diols and 17,20,21-triols, can be detected if the papers are dried[1] and dipped in a freshly prepared mixture of 0.4% tetrazolium blue with 3 N NaOH (1:9); color development is considerably more rapid in the presence of quaternary exchangers (tetraheptylammonium salts or Aliquat) than with salts of TOA, ALA-2 or XLA-3 present. Detection of the 17,20,21-triols has been described[2] previously. The 20,21-diols were revealed as blue spots by spraying a paper

with periodic acid[2], placing it between glass plates for 10 min, allowing it to dry in air, and then dipping it into the alkaline tetrazolium blue solution. For papers lacking an ion exchanger the intensity of color of the spots, particularly those of the 20-oxo-21-glucosiduronates, can be greatly enhanced by spraying with 0.1 N TA·Cl in chloroform before employing the tetrazolium reagent. Also, papers which lack an ion exchanger should be sprayed with the TA·Cl reagent before being treated successively with periodate and tetrazolium blue if 20,21-dihydroxy compounds are to be detected.

RESULTS

Twenty-five monoglucosiduronic acids and three diglucosiduronic acids were chromatographed on paper in duplicate in fifteen chromatography systems. Twelve of these systems consist primarily of chloroform and formamide, with different ion exchangers being added to the mobile phase and an appropriate salt being added to the stationary phase. Three of the solvent combinations are conventional Bush-type systems which have been used previously to chromatograph various steroidal glucosiduronates. Table I lists the glucosiduronic acids which were chromatographed, the solvent systems which were employed, and the abbreviations which are used throughout the text. The compounds are listed in order of decreasing mobility in the presence of TA·Cl.

The R_F and R_M values of the glucosiduronic acids in five systems, each of which employs a different tetraheptylammonium salt, are listed in Table II. Migration of the acids as a group in these systems increases in the following order: $I^- < Br^- < Cl^- < OAc^- < SO_4^{2-}$. The data are displayed in Fig. 1, in which the R_M values found in TA·Cl are plotted along the abscissa and the R_M values found in the presence of the other tetraheptylammonium salts are plotted along the ordinate. The linear relationships shown in Fig. 1 may be expressed by the following regression equations, which were calculated by the least-squares method.

$R_M(TA·I) = 1.05 \, R_M(TA·Cl) + 1.51$ $\quad$ ($n = 7$; $r = 0.987$; $sy·x = 0.039$) $\quad$ (1)

$R_M(TA·Br) = 1.06 \, R_M(TA·Cl) + 0.61$ $\quad$ ($n = 15$; $r = 0.993$; $sy·x = 0.046$) $\quad$ (2)

$R_M(TA·OAc) = 0.80 \, R_M(TA·Cl) - 0.68$ $\quad$ ($n = 21$; $r = 0.991$; $sy·x = 0.057$) $\quad$ (3)

$R_M[(TA)_2·SO_4] = 0.50 \, R_M(TA·Cl) - 0.88$ ($n = 15$; $r = 0.931$; $sy·x = 0.079$) $\quad$ (4)

The equations indicate that, while TA·I and TA·Br are much weaker extractants than TA·Cl (positive intercepts), overall resolution of the acids (in terms of R_M) is very similar with all three of the tetraheptylammonium halides, i.e., the ratio for a/r in eqns. 1 and 2 and in the TA·Cl system is 1.06, 1.07 and 1.00, respectively. As indicated by the intercepts, TA·I and TA·Br are applicable to the chromatography on paper of conjugates which tend to move too far in the TA·Cl system. However, values for a/r in eqns. 3 and 4 indicate that resolution is much poorer in the presence of TA·OAc and $(TA)_2·SO_4$ than in TA·Cl. Glucosiduronic acids migrate at different absolute rates in the different systems for two reasons: (1) differences in the energy with which an anion (I^-, Br^-, etc.) is bound in the chloroform and the formamide phase, and (2) differences in the ability of the anionic moiety of the ion pairs to serve as a proton acceptor in forming a hydrogen bond with a hydroxyl group[2] in the chloroform phase.

TABLE I

LIST OF STEROIDAL GLUCOSIDURONIC ACIDS CHROMATOGRAPHED AND SOLVENT SYSTEMS IN WHICH THEY WERE CHROMATOGRAPHED

A. Steroidal glucosiduronic acids

No.	Compound
	Monoglucosiduronic acids
1	3,20-Dioxopregn-4-en-21-yl β-D-glucopyranosiduronic acid
2	17-Hydroxy-3,20-dioxopregn-4-en-21-yl β-D-glucopyranosiduronic acid
3	21-Hydroxy-20-oxo-5β-pregnan-3α-yl β-D-glucopyranosiduronic acid
4	3α-Hydroxy-20-oxo-5β-pregnan-21-yl β-D-glucopyranosiduronic acid
5	3,11,20-Trioxopregn-4-en-21-yl β-D-glucopyranosiduronic acid
6	11β-Hydroxy-3,20-dioxopregn-4-en-21-yl β-D-glucopyranosiduronic acid
7	17-Hydroxy-3,11,20-trioxopregn-4-en-21-yl β-D-glucopyranosiduronic acid
8	3α,17-Dihydroxy-20-oxo-5β-pregnan-21-yl β-D-glucopyranosiduronic acid
9	11β,18-Epoxy-21-hydroxy-3,20-dioxopregn-4-en-18ξ-yl α-D-glucopyranosiduronic acid
10	3α-Hydroxy-11,20-dioxo-5β-pregnan-21-yl β-D-glucopyranosiduronic acid
11	21-Hydroxy-11,20-dioxo-5β-pregnan-3α-yl β-D-glucopyranosiduronic acid
12	11β,17-Dihydroxy-3,20-dioxopregn-4-en-21-yl β-D-glucopyranosiduronic acid
13	17,21-Dihydroxy-20-oxo-5β-pregnan-3α-yl β-D-glucopyranosiduronic acid
14	3α,11β-Dihydroxy-20-oxo-5β-pregnan-21-yl β-D-glucopyranosiduronic acid
15	3α,17-Dihydroxy-11,20-dioxo-5β-pregnan-21-yl β-D-glucopyranosiduronic acid
16	17,21-Dihydroxy-11,20-dioxo-5β-pregnan-3α-yl β-D-glucopyranosiduronic acid
17	20β,21-Dihydroxy-11-oxo-5β-pregnan-3α-yl β-D-glucopyranosiduronic acid
18	11β,21-Dihydroxy-20-oxo-5β-pregnan-3α-yl β-D-glucopyranosiduronic acid
19	20α,21-Dihydroxy-11-oxo-5β-pregnan-3α-yl β-D-glucopyranosiduronic acid
20	3α,11β,17-Trihydroxy-20-oxo-5β-pregnan-21-yl β-D-glucopyranosiduronic acid
21	17,20β,21-Trihydroxy-11-oxo-5β-pregnan-3α-yl β-D-glucopyranosiduronic acid
22	11β,17,21-Trihydroxy-20-oxo-5β-pregnan-3α-yl β-D-glucopyranosiduronic acid
23	17,20α,21-Trihydroxy-11-oxo-5β-pregnan-3α-yl β-D-glucopyranosiduronic acid
24	11β,17,20β,21-Tetrahydroxy-5β-pregnan-3α-yl β-D-glucopyranosiduronic acid
25	11β,17,20α,21-Tetrahydroxy-5β-pregnan-3α-yl β-D-glucopyranosiduronic acid
	Diglucosiduronic acids
26	20-Oxo-5β-pregnan-3α,21-ylene di(β-D-glucopyranosiduronic acid)
27	11,20-Dioxo-5β-pregnan-3α,21-ylene di(β-D-glucopyranosiduronic acid)
28	11β-Hydroxy-20-oxo-5β-pregnan-3α,21-ylene di(β-D-glucopyranosiduronic acid)

B. Chromatographic systems

| Abbreviation	Components of system
TA·I	0.1 N tetraheptylammonium iodide in $CHCl_3$/0.1 N KI in formamide
TA·Br	0.1 N tetraheptylammonium bromide in $CHCl_3$/0.1 N KBr in formamide
TA·Cl	0.1 N tetraheptylammonium chloride in $CHCl_3$/0.1 N KCl in formamide
TA·OAc	0.1 N tetraheptylammonium acetate in $CHCl_3$/0.1 N $KC_2H_3O_2$ in formamide
(TA)$_2$·SO$_4$	0.1 N tetraheptylammonium sulfate in $CHCl_3$/0.1 N $(NH_4)_2SO_4$ in formamide
Aliquat	0.1 N methyltricaprylylammonium chloride in $CHCl_3$/0.1 N KCl in formamide
TOA·HCl	0.1 N tri-n-octylamine hydrochloride in $CHCl_3$/0.1 N KCl in formamide
ALA-2·HCl	0.1 N Amberlite LA-2 hydrochloride in $CHCl_3$/0.1 N KCl in formamide
XLA-3·HCl	0.1 N Amberlite XLA-3 hydrochloride in $CHCl_3$/0.1 N KCl in formamide
(TOA·H)$_2$SO$_4$	0.1 N tri-n-octylamine hydrosulfate in $CHCl_3$/0.1 N $(NH_4)_2SO_4$ in formamide
(ALA-2·H)$_2$SO$_4$	0.1 N Amberlite LA-2 hydrosulfate in $CHCl_3$/0.1 N $(NH_4)_2SO_4$ in formamide
(XLA-3·H)$_2$SO$_4$	0.1 N Amberlite XLA-3 hydrosulfate in $CHCl_3$/0.1 N $(NH_4)_2SO_4$ in formamide
Dichloroethane[15]	1,2-Dichloroethane–*tert.*-butanol–water–acetic acid (75:25:70:30)
Butyl acetate[16]	Butyl acetate–n-butanol–water–acetic acid (8:2:9:1)
Butanol	n-Butanol–water (1:1)
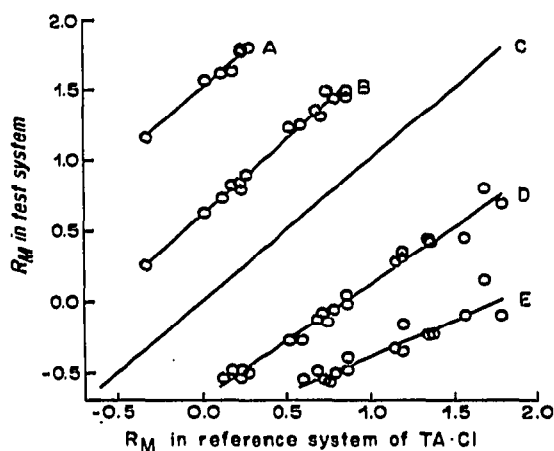
Fig. 1. Comparison of the chromatographic migration (R_M scale) of steroidal glucosiduronic acids in the presence of various tetraheptylammonium salts with migration in the reference system of TA·Cl; data from Table II. A = TA·I vs. TA·Cl; B = TA·Br vs. TA·Cl; C = TA·Cl vs. TA·Cl; D = TA·OAc vs. TA·Cl; E = (TA)₂·SO₄ vs. TA·Cl. See eqns. 1–4 in text; components of solvent systems are given in Table I.

TABLE II

R_F AND R_M VALUES OF STEROIDAL GLUCOSIDURONIC ACIDS IN THE PRESENCE OF VARIOUS TETRAHEPTYLAMMONIUM SALTS

Compound*	Solvent system*									
	TA·I		TA·Br		TA·Cl		TA·OAc		(TA)₂·SO₄	
	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
1	0.065	1.16	0.36	0.25	0.68	−0.33	0.88	—	0.89	—
2	0.027	1.56	0.19	0.63	0.49	0.02	0.83	—	0.86	—
3	0.024	1.61	0.16	0.72	0.43	0.12	0.77	−0.53	0.85	—
4	0.023	1.63	0.13	0.83	0.40	0.18	0.75	−0.48	0.86	—
5	0.017	1.76	0.14	0.79	0.37	0.23	0.75	−0.48	0.85	—
6	0.016	1.79	0.13	0.83	0.37	0.23	0.77	−0.53	0.86	—
7	0.016	1.79	0.11	0.91	0.35	0.27	0.76	−0.50	0.84	—
8	—	—	0.056	1.23	0.23	0.53	0.65	−0.27	0.82	—
9	—	—	0.053	1.25	0.20	0.60	0.65	−0.27	0.78	−0.55
10	—	—	0.043	1.35	0.17	0.69	0.57	−0.12	0.75	−0.48
11	—	—	0.045	1.33	0.16	0.72	0.55	−0.09	0.77	−0.53
12	—	—	0.031	1.50	0.15	0.75	0.58	−0.14	0.78	−0.55
13	—	—	0.034	1.45	0.14	0.79	0.53	−0.05	0.76	−0.50
14	—	—	0.031	1.50	0.12	0.87	0.48	0.04	0.71	−0.39
15	—	—	0.034	1.45	0.12	0.87	0.51	−0.02	0.75	−0.48
16	—	—	0.013	1.88	0.066	1.15	0.35	0.27	0.68	−0.33
17	—	—	—	—	0.060	1.20	0.33	0.31	0.69	−0.35
18	—	—	—	—	0.059	1.20	0.31	0.35	0.59	−0.16
19	—	—	—	—	0.042	1.36	0.26	0.45	0.63	−0.23
20	—	—	—	—	0.041	1.37	0.27	0.43	0.63	−0.23
21	—	—	—	—	0.026	1.57	0.26	0.45	0.56	−0.10
22	—	—	—	—	0.020	1.69	0.14	0.79	0.41	0.16
23	—	—	—	—	0.016	1.79	0.17	0.69	0.55	−0.09
24	—	—	—	—	—	—	0.10	0.95	0.40	0.18
25	—	—	—	—	—	—	0.044	1.34	0.28	0.41
26	—	—	—	—	0.036	1.43	0.45	0.09	0.83	—
27	—	—	—	—	0.016	1.79	0.24	0.50	0.78	−0.55
28	—	—	—	—	—	—	0.16	0.72	0.69	−0.35

* See Table I for list of glucosiduronic acids and solvent systems.

Poorer resolution of the acids with the stronger extractants, TA·OAc and $(TA)_2 \cdot SO_4$, may be explained in part by this difference in hydrogen bonding ability. As hydrogen bonding becomes more extensive in proceeding from I^- to SO_4^{2-}, the solubility of the bonded hydroxyl group in the mobile phase increases and the magnitude of the ΔR_{M_0} value for the hydroxyl group decreases. Thus, overall resolution for the series of solutes is diminished as partition of a specific group into the mobile phase increases.

In Table III, the R_F and R_M values are listed for the acids in four solvent systems each of which employs a different substituted ammonium chloride in the mobile phase. The following equations relate the R_M values of these solvent systems to those of the TA·Cl system which are given in Table II.

$R_M(Aliquat) = 0.96\ R_M(TA \cdot Cl) - 0.05$ ($n = 23$; $r = 0.996$; $sy \cdot x = 0.053$) (5)

$R_M(TOA \cdot HCl) = 1.28\ R_M(TA \cdot Cl) + 0.17$ ($n = 18$; $r = 0.970$; $sy \cdot x = 0.145$) (6)

$R_M(ALA\text{-}2 \cdot HCl) = 1.26\ R_M(TA \cdot Cl) + 0.18$ ($n = 17$; $r = 0.970$; $sy \cdot x = 0.136$) (7)

$R_M(XLA\text{-}3 \cdot HCl) = 1.28\ R_M(TA \cdot Cl) - 0.03$ ($n = 18$; $r = 0.983$; $sy \cdot x = 0.110$) (8)

TABLE III

R_F AND R_M VALUES OF STEROIDAL GLUCOSIDURONIC ACIDS IN SYSTEMS WHICH CONTAIN A SUBSTITUTED AMMONIUM CHLORIDE

Compound*	Solvent system*							
	Aliquat		TOA·HCl		ALA-2·HCl		XLA-3·HCl	
	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
1	0.68	−0.33	0.65	−0.27	0.66	−0.29	0.76	−0.50
2	0.53	−0.05	0.33	0.31	0.34	0.29	0.50	0.00
3	0.42	0.14	0.46	0.07	0.43	0.12	0.39	0.19
4	0.45	0.09	0.34	0.29	0.35	0.27	0.45	0.09
5	0.41	0.16	0.29	0.39	0.31	0.35	0.40	0.18
6	0.40	0.18	0.26	0.45	0.21	0.58	0.33	0.31
7	0.39	0.19	0.14	0.79	0.16	0.72	0.29	0.39
8	0.29	0.39	0.092	0.99	0.093	0.99	0.17	0.69
9	0.23	0.53	0.12	0.87	0.11	0.91	0.23	0.53
10	0.19	0.63	0.071	1.12	0.081	1.06	0.14	0.79
11	0.17	0.69	0.095	0.98	0.084	1.04	0.081	1.06
12	0.19	0.63	0.052	1.26	0.047	1.31	0.11	0.91
13	0.16	0.72	0.063	1.17	0.066	1.15	0.062	1.18
14	0.15	0.75	0.060	1.20	0.048	1.30	0.083	1.04
15	0.16	0.72	0.037	1.42	0.037	1.42	0.073	1.10
16	0.071	1.12	0.019	1.71	0.023	1.63	0.029	1.53
17	0.068	1.14	0.016	1.79	0.014	—	0.012	—
18	0.066	1.15	0.035	1.44	0.034	1.45	0.031	1.50
19	0.043	1.35	0.010	—	—	—	—	—
20	0.062	1.18	0.014	—	0.012	—	0.028	1.54
21	0.032	1.48	—	—	—	—	—	—
22	0.022	1.65	—	—	—	—	—	—
23	0.025	1.59	—	—	—	—	—	—
24	—	—	—	—	—	—	—	—
25	—	—	—	—	—	—	—	—
26	0.046	1.32	0.022	1.65	0.021	1.67	0.037	1.42
27	0.024	1.61	—	—	—	—	0.012	—
28	0.013	—	—	—	—	—	—	—

    * See Table I for list of glucosiduronic acids and solvent systems.

Eqn. 5 indicates that the system which contains Aliquat is nearly identical in proper-
ties to the TA·Cl system; the slope (and also a/r) is 0.96, the intercept -0.05, the
correlation coefficient ≈ 1.0, and the standard error of estimate small (0.053).

Whereas most of the solutes migrate more slowly in the TOA·HCl system
than in the presence of TA·Cl, eqn. 6, with $a/r = 1.32$, shows that the former solvent
system provides generally better resolution. Furthermore, the value for $sy·x$ indicates
that some of the solutes migrate at considerably different rates relative to one another
in the two systems. This difference in mobility is illustrated in Fig. 2, in which R_M(TOA·
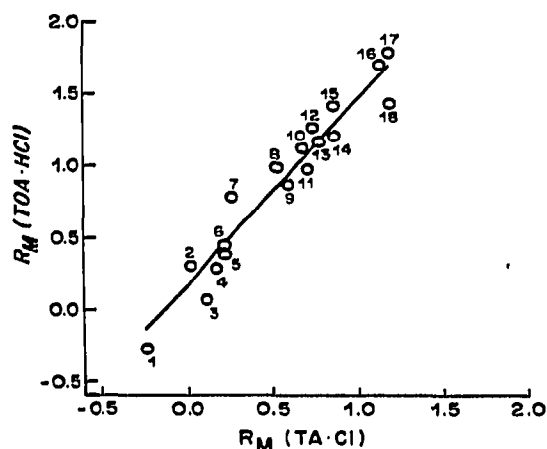HCl) is plotted as ordinate against R_M(TA·Cl).

Fig. 2. Comparison of the chromatographic migration (R_M scale) of steroidal glucosiduronic acids
in the TOA·HCl and TA·Cl systems; data from Tables II and III. See eqn. 6 in text; a list of the glu-
cosiduronic acids and the components of the solvent systems are given in Table I.

As indicated by eqns. 7 and 8, ALA-2·HCl and XLA-3·HCl are quite similar
in properties to TOA·HCl. However, there are a few separations which are unique
to each of these systems which employs an amine hydrochloride.

R_F and R_M values for the acids in the presence of three amine hydrosulfates
are listed in Table IV. The relationship of these systems to the TA·Cl system follows.

$$R_M[(\text{TOA}·\text{H})_2\text{SO}_4] = 1.17\ R_M(\text{TA}·\text{Cl}) - 0.43$$
$$(n = 22;\ r = 0.987;\ sy·x = 0.106) \quad (9)$$
$$R_M[(\text{ALA-2}·\text{H})_2\text{SO}_4] = 1.17\ R_M(\text{TA}·\text{Cl}) - 0.61$$
$$(n = 22;\ r = 0.989;\ sy·x = 0.097) \quad (10)$$
$$R_M[(\text{XLA-3}·\text{H})_2\text{SO}_4] = 0.88\ R_M(\text{TA}·\text{Cl}) - 0.62$$
$$(n = 21;\ r = 0.955;\ sy·x = 0.144) \quad (11)$$

By comparing the intercepts of these equations with those of eqns. 6, 7 and 8, it is
apparent that the migration of the acids as a group is increased markedly by using
an amine hydrosulfate instead of an amine hydrochloride. Furthermore, for the
tertiary amine TOA and the secondary amine ALA-2, resolution is not greatly
diminished in going from the hydrochloride to the hydrosulfate; for TOA a/r decreases

TABLE IV

R_F AND R_M VALUES OF STEROIDAL GLUCOSIDURONIC ACIDS IN SYSTEMS WHICH CONTAIN AMINE HYDROSULFATES

Compound*	Solvent system*					
	$(TOA \cdot H)_2SO_4$		$(ALA-2 \cdot H)_2SO_4$		$(XLA-3 \cdot H)_2SO_4$	
	R_F	R_M	R_F	R_M	R_F	R_M
1	0.81	—	0.84	—	0.88	—
2	0.67	—0.39	0.74	—0.45	0.80	—
3	0.72	—0.41	0.74	—0.45	0.73	—0.43
4	0.64	—0.25	0.73	—0.43	0.78	—0.55
5	0.63	—0.23	0.71	—0.39	0.71	—0.39
6	0.59	—0.16	0.66	—0.29	0.70	—0.37
7	0.45	0.09	0.63	—0.23	0.67	—0.31
8	0.31	0.35	0.51	—0.02	0.64	—0.25
9	0.43	0.12	0.58	—0.14	0.71	—0.39
10	0.28	0.41	0.42	0.14	0.52	—0.04
11	0.35	0.27	0.39	0.19	0.38	0.21
12	0.23	0.53	0.33	0.31	0.46	0.07
13	0.22	0.55	0.35	0.27	0.45	0.09
14	0.23	0.53	0.28	0.41	0.43	0.12
15	0.15	0.75	0.27	0.43	0.48	0.04
16	0.090	1.00	0.12	0.87	0.21	0.58
17	0.089	1.01	0.13	0.83	0.27	0.43
18	0.14	0.79	0.15	0.75	0.19	0.63
19	0.075	1.09	0.089	1.01	0.21	0.58
20	0.058	1.21	0.091	1.00	0.28	0.41
21	0.034	1.45	0.062	1.18	0.17	0.69
22	0.025	1.59	0.025	1.59	0.069	1.13
23	0.023	1.63	0.045	1.33	0.15	0.75
24	—	—	0.018	1.74	0.071	1.12
25	—	—	—	—	0.050	1.28
26	0.18	0.66	0.44**	0.10	0.54	—0.07
27	0.055	1.24	0.18**	0.66	0.35	0.27
28	0.029	1.53	0.063**	1.17	0.20	0.60

* See Table I for list of glucosiduronic acids and solvent systems.
** Streaking.

from 1.32 to 1.19, and for ALA-2 from 1.30 to 1.18. However, resolution with the primary amine hydrosulfate $(XLA-3 \cdot H)_2SO_4$ is considerably poorer than with $XLA-3 \cdot HCl$, the values for a/r being 0.92 and 1.30, respectively.

In Table V, the R_F and R_M values for the acids in three conventional chromatography systems are listed and the following equations relate these systems to the standard system.

$$R_M(\text{Dichloroethane}) = 0.60 \ R_M(\text{TA} \cdot \text{Cl}) - 0.29$$
$$(n = 23; \ r = 0.869; \ sy \cdot x = 0.201) \quad (12)$$
$$R_M(\text{Butyl acetate}) = 0.27 \ R_M(\text{TA} \cdot \text{Cl}) - 0.11$$
$$(n = 23; \ r = 0.636; \ sy \cdot x = 0.193) \quad (13)$$
$$R_M(\text{Butanol}) = 0.23 \ R_M(\text{TA} \cdot \text{Cl}) + 0.01 \quad (n = 23; \ r = 0.624; \ sy \cdot x = 0.168) \quad (14)$$

TABLE V

R_F AND R_M VALUES OF STEROIDAL GLUCOSIDURONIC ACIDS IN BUSH-TYPE SYSTEMS

Compound*	Solvent system*					
	Dichloroethane		Butyl acetate		Butanol	
	R_F	R_M	R_F	R_M	R_F	R_M
1	0.77	−0.53	0.61	−0.19	0.51	−0.02
2	0.55	−0.09	0.53	−0.05	0.47	0.05
3	0.77	−0.53	0.69	−0.35	0.59	−0.16
4	0.70	−0.37	0.66	−0.29	0.59	−0.16
5	0.62	−0.21	0.43	0.12	0.32	0.33
6	0.50	0.00	0.45	0.09	0.39	0.19
7	0.38	0.21	0.35	0.27	0.36	0.25
8	0.41	0.16	0.57	−0.12	0.54	−0.07
9	0.54	−0.07	0.30	0.37	0.21	0.58
10	0.50	0.00	0.47	0.05	0.44	0.10
11	0.55	−0.09	0.48	0.04	0.39	0.19
12	0.23	0.53	0.34	0.29	0.37	0.23
13	0.48	0.04	0.59	−0.16	0.52	−0.04
14	0.39	0.19	0.50	0.00	0.45	0.09
15	0.25	0.48	0.41	0.16	0.44	0.10
16	0.28	0.41	0.43	0.12	0.32	0.33
17	0.31	0.35	0.37	0.23	0.37	0.23
18	0.45	0.09	0.54	−0.07	0.38	0.21
19	0.28	0.41	0.37	0.23	0.35	0.27
20	0.14	0.79	0.37	0.23	0.37	0.23
21	0.15	0.75	0.23	0.53	0.27	0.43
22	0.16	0.72	0.42	0.14	0.27	0.43
23	0.14	0.79	0.18	0.66	0.19	0.63
24	0.079	1.07	0.15	0.75	0.19	0.63
25	0.062	1.18	0.15	0.75	0.14	0.79
26	0.067	1.14	0.11	0.91	0.041**	1.37
27	0.024	1.61	0.035	1.44	0.014	—
28	0.016	1.79	0.039	1.39	—	—

    * See Table I for list of glucosiduronic acids and solvent systems.
    ** Streaking.

As judged by the values for a/r (namely, 0.69, 0.42 and 0.37, respectively), these systems spread the compounds out considerably less than does the standard system. When judged by the correlation coefficients and $sy·x$ values, it is apparent that the resolving properties of these systems are markedly different from those of the standard system. On this basis, they have high discriminating power for pairs of compounds that are not discriminated by the TA·Cl system, nor by other solvent systems which have high correlation coefficients with the standard one*. Furthermore, the following

    * It follows, also, that the standard system has high discriminating power for pairs of compound that are not discriminated by either the dichloroethane system, the butyl acetate system, or the butanol system.

equations show that two of the three Bush-type systems have low correlation coefficients when they are compared to one another.

R_M(Dichloroethane) = 1.31 R_M(Butyl acetate) + 0.05

$$(n = 25; \ r = 0.841; \ sy \cdot x = 0.256) \quad (15)$$

R_M(Dichloroethane) = 1.39 R_M(Butanol) — 0.07

$$(n = 25; \ r = 0.743; \ sy \cdot x = 0.316) \quad (16)$$

R_M(Butyl acetate) = 1.10 R_M(Butanol) — 0.11

$$(n = 25; \ r = 0.925; \ sy \cdot x = 0.114) \quad (17)$$

Decreased correlation coefficients, *i.e.*, greater scatter of R_M values when R_M(Y) is plotted *vs.* R_M(X) as a standard, are to be expected when there are major differences in the manner or extent to which the components of two chromatography systems react with one or more functions of molecules in a group of compounds. Leo *et al.* discuss this general phenomenon[7] and, with certain solvent systems, derive one regression equation for "H-donor solutes", another for "H-acceptor solutes", and a third equation for "neutral solutes".

When a group of glucusiduronic acids was chromatographed in the presence of three different concentrations of TA·Cl (namely, 0.025 N, 0.050 N, and 0.10 N; all in the presence of 0.10 N KCl), the following relationships were found.

R_M(0.025 N TA·Cl) = 1.20 R_M(0.10 N TA·Cl) + 0.60

$$(n = 15; \ r = 0.994; \ sy \cdot x = 0.050) \quad (18)$$

R_M(0.050 N TA·Cl) = 1.14 R_M(0.10 N TA·Cl) + 0.25

$$(n = 15; \ r = 0.996; \ sy \cdot x = 0.039) \quad (19)$$

Analogous results were obtained by using three concentrations of TA·OAc with 0.1 N KOAc.

R_M(0.025 N TA·OAc) = 1.25 R_M(0.10 N TA·OAc) + 0.82

$$(n = 15; \ r = 0.979; \ sy \cdot x = 0.088) \quad (20)$$

R_M(0.050 N TA·OAc) = 1.06 R_M(0.10 N TA·OAc) + 0.39

$$(n = 15; \ r = 0.992; \ sy \cdot x = 0.045) \quad (21)$$

With both TA·Cl and TA·OAc, the value for a/r (resolving power) decreased as the concentration of exchanger was raised to increase mobility. This influence is a type of phenomenon that is common to most isoprotic chromatography systems[12]; decreased resolving power is associated[13] with increased mobility. In this particular case, we postulate that the decreased resolving power is due to increased hydrogen bonding[2] with nonglucuronyl hydroxyl groups in the conjugates as the concentration of the TA·Cl or TA·OAc increases.

The following results were obtained with three concentrations of TOA·HCl, a weak hydrogen bonder, in the presence of 0.10 N KCl.

R_M(0.025 N TOA·HCl) = 0.94 R_M(0.10 N TOA·HCl) + 0.46

$$(n = 14; \ r = 0.993; \ sy \cdot x = 0.058) \quad (22)$$

R_M(0.050 N TOA·HCl) = 0.99 R_M(0.10 N TOA·HCl) + 0.18

$$(n = 14; \ r = 0.997; \ sy \cdot x = 0.036) \quad (23)$$

TABLE VI

ΔR_{Mg} VALUES FOR GROUPS IN VARIOUS CHROMATOGRAPHY SYSTEMS

Values from data in Tables II–V.

Compound pairs[*]		Chromatographic system[*]									
		Butyl acetate	$(TA)_2 \cdot SO_4$	Dichloro-ethane	$TA \cdot OAc$	$(XLA \cdot 2 \cdot H)_2 SO_4$	$TA \cdot Cl$	$TA \cdot Br$	$(ALA \cdot 2 \cdot H)_2 SO_4$	$(TOA \cdot H)_2 SO_4$	$TOA \cdot HCl$
A. 11β-Hydroxyl											
Conjugated at C-3	18– 3	0.28	—	0.62	0.88	1.06	1.08	—	1.20	1.20	1.37
	22–13	0.30	0.66	0.68	0.84	1.04	0.90	—	1.32	1.04	—
	Mean	0.29	—	0.65	0.86	1.05	0.99	—	1.26	1.12	—
Conjugated at C-21	6– 1	0.28	—	0.53	—	—	0.56	0.58	—	—	0.72
	12– 2	0.34	—	0.62	—	—	0.73	0.87	0.76	0.92	0.95
	14– 4	0.29	—	0.56	0.52	0.67	0.69	0.67	0.84	0.78	0.91
	20– 8	0.35	—	0.63	0.70	0.66	0.84	—	1.02	0.86	—
	Mean	0.32	—	0.59	0.61	0.67	0.71	0.71	0.87	0.85	0.86
Conjugated at C-3 and C-21	28–26	0.48	—	0.65	0.63	0.67	—	—	1.07	0.87	—
B. 17α-Hydroxyl											
Conjugated at C-3	13– 3	0.19	—	0.57	0.48	0.52	0.67	0.73	0.72	0.96	1.10
	16–11	0.08	0.20	0.50	0.36	0.37	0.43	—	0.68	0.73	0.73
	22–18	0.21	0.32	0.63	0.44	0.50	0.49	—	0.84	0.80	—
	21–17	0.30	0.25	0.40	0.14	0.26	0.37	—	0.35	0.44	—
	23–19	0.43	0.14	0.38	0.24	0.17	0.43	—	0.32	0.54	—
	Mean	0.24	0.23	0.50	0.33	0.36	0.48	—	0.58	0.69	—

Conjugated at C-21										
2– 1	0.14	—	0.44	—	—	0.35	0.38	—	—	0.58
8– 4	0.17	—	0.53	0.21	0.30	0.35	0.40	0.41	0.60	0.70
7– 5	0.15	—	0.42	−0.02	0.08	0.04	0.12	0.16	0.32	0.40
12– 6	0.20	—	0.53	0.39	0.44	0.52	0.67	0.60	0.69	0.81
15–10	0.11	0.00	0.48	0.10	0.08	0.18	0.10	0.29	0.34	0.30
20–14	0.23	0.16	0.60	0.39	0.28	0.50	—	0.59	0.68	—
Mean	0.17	—	0.50	0.21	0.24	0.32	0.33	0.41	0.53	0.56
C. II-Ketone										
Conjugated at C-3										
11– 3	0.39	—	0.44	0.44	0.64	0.60	0.61	0.64	0.68	0.91
16–13	0.28	0.17	0.37	0.32	0.49	0.36	—	0.60	0.45	0.54
Mean	0.34	—	0.41	0.38	0.57	0.48	—	0.62	0.57	0.73
Conjugated at C-21										
5– 1	0.31	—	0.32	—	—	0.56	0.54	—	—	0.66
7– 2	0.32	—	0.30	—	—	0.25	0.28	0.22	0.48	0.48
10– 4	0.34	—	0.37	0.36	0.51	0.51	0.52	0.57	0.66	0.83
15– 8	0.28	—	0.32	0.25	0.29	0.34	0.22	0.45	0.40	0.43
Mean	0.31	—	0.33	0.31	0.40	0.42	0.39	0.41	0.51	0.60
Conjugated at C-3 and C-21										
27–26	0.53	—	0.47	0.41	0.34	0.36	—	0.56	0.58	—

* See Table I for list of glucosiduronic acids and chromatographic systems.

TABLE VII

ΔR_M VALUES FOR CONVERSION OF KETONES TO HYDROXYLS IN VARIOUS CHROMATOGRAPHY SYSTEMS

Values from data in Tables II–V.

Compound pairs*	Chromatographic system*									
	Butyl acetate	(TA)₂SO₄	Dichloro-ethane	TA·OAc	(XLA-3·H)₂SO₄	TA·Cl	TA·Br	(ALA-2·H)₂SO₄	(TOA·H)₂SO₄	TOA·HCl
A. 11β-Hydroxyl vs. 11-ketone										
Conjugated at C-3 18-11	-0.11	0.37	0.18	0.44	0.42	0.48	—	0.56	0.52	0.46
22-16	0.02	0.49	0.31	0.52	0.55	0.54	—	0.72	0.59	—
24-21	0.22	0.28	0.32	0.50	0.43	—	—	0.56	—	—
25-23	0.09	0.50	0.39	0.65	0.53	—	—	—	—	—
Mean	0.06	0.41	0.30	0.53	0.48	—	—	0.61	—	—
Conjugated at C-21 6-5	-0.03	—	0.21	-0.05	0.02	0.00	0.04	0.10	0.07	0.06
12-7	0.02	—	0.32	0.36	0.38	0.48	0.59	0.54	0.44	0.47
14-10	-0.05	0.09	0.19	0.16	0.16	0.18	0.15	0.27	0.12	0.08
20-15	0.07	0.25	0.31	0.45	0.37	0.50	—	0.57	0.46	—
Mean	0.00	—	0.26	0.23	0.23	0.29	0.26	0.37	0.27	0.20
Conjugated at C-3 and C-21 28-27	-0.05	0.20	0.18	0.22	0.33	—	—	0.51	0.29	—
B. 5β:3α-Hydroxyl vs. Δ⁴:3-ketone										
Conjugated at C-21 4-1	-0.10	—	0.16	—	—	0.51	0.58	—	—	0.56
8-2	-0.07	—	0.25	—	—	0.51	0.60	0.43	0.74	0.68
10-5	-0.07	—	0.21	0.36	0.35	0.46	0.56	0.53	0.64	0.73
14-6	-0.09	—	0.19	0.57	0.49	0.64	0.67	0.60	0.69	0.75
15-7	-0.09	—	0.27	0.48	0.35	0.60	0.54	0.66	0.66	0.63
20-12	-0.32	0.32	0.26	0.57	0.34	0.62	—	0.69	0.68	—
Mean	-0.08	—	0.22	0.50	0.38	0.56	0.59	0.58	0.68	0.67
C. 20β-Hydroxyl vs. 20-ketone										
Conjugated at C-3 17-11	0.19	0.18	0.44	0.40	0.22	0.48	—	0.64	0.74	0.81
21-16	0.41	0.23	0.34	0.18	0.11	0.42	—	0.31	0.45	—
24-22	0.61	0.02	0.35	0.16	-0.01	—	—	0.15	—	—
Mean	0.40	0.14	0.38	0.25	0.11	0.45	—	0.37	0.60	—
D. 20α-Hydroxyl vs. 20-ketone										
Conjugated at C-3 19-11	0.19	0.30	0.50	0.54	0.37	0.64	—	0.82	0.82	—
23-16	0.54	0.24	0.38	0.42	0.17	0.64	—	0.46	0.63	—
25-22	0.61	0.25	0.46	0.55	0.15	—	—	—	—	—
Mean	0.45	0.26	0.45	0.50	0.23	0.64	—	0.64	0.73	—

* See Table I for list of glucosiduronic acids and chromatographic systems.

Values for a/r do not decrease with increasing concentration of exchanger. We interpret these findings to represent insignificant hydrogen bonding[2] of nonglucuronyl hydroxyl groups by TOA·HCl: the increase in mobility with increase in TOA·HCl concentration is assumed to be due almost exclusively to interaction with the carboxyl group, and attendant shielding of the hydroxyl groups on the glucuronyl moiety of the conjugate.

In the foregoing equations the value for a/r, and for slope alone in instances when $r \approx 1.00$, has been interpreted as a measure of general resolving ability (in terms of R_M) of a chromatography system for the group of acids being studied. If this interpretation is correct, there should be a relationship between values for a/r of an equation and the magnitude of values* indicating ΔR_{M_a} and ΔR_{M_r} of the polar functions in the molecules. As shown in Tables VI and VII, in which the systems are listed in the order of increasing slope relative to the standard system, there is a general trend toward increasing values for ΔR_M with increasing slope. Since the total R_M of a compound is made up of increments of R_M for each functional group and since the ΔR_M values of each functional group vary somewhat with the exchanger used, it is not surprising that the ΔR_M values for a particular functional group are not exactly parallel with the slopes of the regression equations. However, if the mean of all ΔR_M values which are given in Tables VI and VII is obtained, it is apparent that there is good general agreement between ΔR_M values and values for a/r (Table VIII): values for slope and for a/r increase in parallel.

The compounds in Tables VI and VII are grouped as (1) C-3 conjugates and (2) C-21 conjugates, because in general ΔR_M values for the polar functional groups are larger for the C-3 conjugates than for the C-21 analogues, especially when liquid ion exchangers are employed. The exchangers have long chains which might be expected to exert a shielding effect far beyond the locus of their primary action, namely, the carboxyl group. Also, there appears to be some interaction between the 11-oxo group and the 17-hydroxyl group when both of these functions are present in a compound and when a liquid ion exchanger is employed: The ΔR_M value for 17-hydroxyl is abnormally small when an 11-oxo group is present (Table VI, see values for 7-5, 15-10, and 16-11) and the ΔR_M value for 11-oxo is anomalously low when a 17-hydroxyl group is in the molecule (see 7-2, 15-8, and 16-13).

DISCUSSION

If the foregoing equations are to express the relationship between the chromatographic properties of the standard system and the various other solvent systems relative to a new group of steroidal glucosiduronic acids it will be necessary that the properties of the new group of conjugates be typical of the group of compounds for which the equations are derived. An example of the nonhomogeneity of the present group of compounds can be observed in Fig. 2, in which R_M values of the acids in TOA·HCl are plotted as ordinate against R_M values of the acids in TA·Cl; conjugates with a hydroxyl function at C-17 tend to fall above the line; those with hydrogen at C-17 tend to fall below the line.

* The change in R_M caused by the simple substitution[12] of a group for hydrogen(s) is designated ΔR_{M_a}; the change in R_M caused by a more complicated change in substituents is designated ΔR_{M_r}.

V. R. MATTOX, R. D. LITWILLER, J. E. GOODRICH

TABLE VIII

RELATIONSHIP OF MEAN ΔR_M FOR FUNCTIONAL GROUPS TO SLOPE a AND INTERCEPT b IN THE EQUATION $R_M(Y) = a \cdot R_M(TA \cdot Cl) \div b$

	Solvent system									
	Butyl acetate	$(TA)_2 \cdot SO_4$	Dichloro-ethane	$TA \cdot OAc$	$(XLA-3 \cdot H)_2SO_4$	$TA \cdot Cl$	$TA \cdot Br$	$(ALA-2 \cdot H)_2SO_4$	$(TOA \cdot H)_2SO_4$	$TOA \cdot HCl$
Mean ΔR_M	0.20	0.26	0.40	0.40	0.39	0.50	0.47	0.58	0.62	0.65
Slope a	0.27	0.50	0.60	0.80	0.88	1.00	1.06	1.17	1.17	1.28
a/r	0.42	0.54	0.69	0.81	0.92	1.00	1.07	1.18	1.18	1.32
Intercept b	−0.11	−0.88	−0.29	−0.68	−0.62	0.00	±0.61	−0.61	−0.43	+0.17
N[*]	46	22	46	39	39	40	22	40	38	27

[*] N is the number of values from which the mean was obtained.

If one scans the R_F values in Table II under TA·Cl and compares them with R_F values in Table V under butyl acetate, he is impressed by the fact that many pairs of compounds are resolved far better in the latter system than in the former. However, if one rearranges the compounds in Table I in order of increasing R_F value in butyl acetate (rather than in TA·Cl) and then compares R_F values in butyl acetate sequentially with the R_F values in TA·Cl, he is impressed by the finding that many pairs of compounds are resolved much better in the latter solvent system. The foregoing findings are a consequence of the order of arrangement of the compounds in the tables and the differences in discriminating ability of the two solvent systems for the conjugates.

A ΔR_M value of 0.14 is just adequate for separating two compounds which give reasonably compact spots by paper chromatography when the solvent front has advanced 30 cm and when the mean R_M is 0.00. If the ΔR_M is adequate for potential separation of two compounds but actual migration is too low, the compounds can be separated by overrunning the chromatogram. A mixture of compounds 10, 11, and 12 was resolved in the TOA·HCl system by overrunning for 46 h; compounds 2, 5, and 6 were separated in XLA-3·HCl by overrunning for 6 h; a mixture of compounds 5, 6, and 7 was resolved in ALA-2·HCl by overrunning for 15 h.

Correlation of results from paper chromatography in two solvent systems is simpler when R_M values are used than when R_F values are employed. However, the extent to which two compounds are separated in terms of distance on a chromatogram is easier to visualize when R_F values are used. Fig. 3 shows the relationship between the distance by which a pair of compounds is separated at constant ΔR_M and the mean distance the compounds have migrated (in R_M and in R_F units). The figure is

Fig. 3. Relationship between the mean migration of a pair of compounds which are separated by 0.20 R_M unit and the distance by which the compounds are separated if the solvent front is allowed to advance to 30 cm beyond the origin.

based on a separation of two substances by 0.20 R_M unit and a solvent front of 30 cm. Starting with a mean R_M of $+$ 1.80, it is apparent that the distance between the centers of two spots representing the two compounds increases as the mean R_M for the pair of compounds decreases progressively until maximal separation is achieved at a mean R_M value of 0.00 (R_F = 0.50). The extent of separation then decreases progressively as the mean R_M value becomes more negative (R_M approaches $-\infty$; R_F approaches 1.00). Thus, when choosing a system for separating a pair of compounds, it is important to consider the area of the chromatogram in which the compounds will migrate in the various systems as well as the relative overall resolution as reflected by values for a/r or specified by magnitude of ΔR_M value. This general problem has been discussed by Bush from a somewhat different[14] point of view.
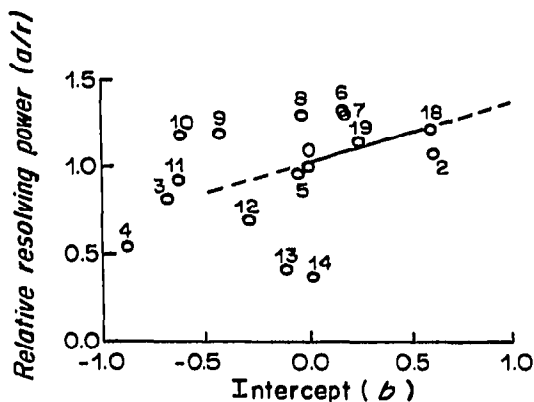
Fig. 4. Relationship between the relative resolving power of solvent systems (a/r) and polarity (intercept b). The point designated 0 represents the reference system (TA·Cl); numerals adjacent to the circles refer to the equations from which the data were derived.

The relationship between relative resolving power (a/r) and polarity (intercept b) for the solvent systems given in this paper is shown in Fig. 4. The line connecting points 18, 19, and 0 illustrates the decrease in resolving power with change in b caused by an increase in the concentration of an ion exchanger, TA·Cl, which has a strong tendency to form hydrogen bonds. The abscissa may be considered to be a "polarity scale" for the series of conjugates relative to migration in the reference system (TA·Cl). For example, compounds for which R_M in the TA·Cl system is about 0.50 would appear on the scale in the vicinity of -0.50. On the whole, resolution of compounds for which R_M in the reference system is roughly 0.50 is better in the presence of either (TOA·H)$_2$SO$_4$ (eqn. 9) or (ALA-2·H)$_2$SO$_4$ (eqn. 10) because these systems have high values for a/r and, in addition, the compounds of interest are likely to migrate near the center of the chromatogram where maximal resolution is achieved. Similar generalizations can be made concerning the relative resolving ability of the various systems for groups of solutes of widely different polarity relative to migration in the reference system.

Correlation of chromatographic data by means of regression equations should facilitate the following:

(1) The selection of a solvent system of the appropriate polarity.

(2) The selection of a chromatography system which has good resolving power (large value for a/r relative to this ratio for other systems available).

(3) Choosing consecutive systems in which the probability is large that a pair of compounds will be discriminated (use of two solvent systems which are related mathematically to one another by an equation which has a small correlation coefficient).

(4) Correlation of partition-type data which have been obtained by different techniques such as paper chromatography, countercurrent distribution, thin-layer chromatography, partition chromatography on columns, etc.

ACKNOWLEDGEMENTS

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