Journal of Chromatography, 212 (1981) 139–152
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,808

THE ROLE OF HYDROGEN BONDS IN CHROMATOGRAPHY

I. AMINO- AND HYDROXYANTHRAQUINONES

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(Received March 17th, 1981)

SUMMARY

The effect of hydrogen bonds on chromatographic separations is demonstrated on the example of amino- and hydroxyanthraquinones and their O- and N-methyl derivatives. The R_E values were used to choose a set of new stationary phases and to demonstrate the dependence between the shift in the R_F values for compounds with hydrogen bonds and the overall energy of the hydrogen bonds of all the donor and acceptor groups in the corresponding stationary phase.

INTRODUCTION

The importance of hydrogen bonds, both intermolecular and intramolecular, in chromatographic separations, especially in paper and thin-layer chromatography, is well established. Most workers, however, consider only the qualitative side of this phenomenon and point out the shift in R_F values on formation on internal hydrogen bonds compared with isomeric compounds that do not contain this type of bond[1–4].

It is obvious that the energies of these hydrogen bonds differ, depending on the nature of the atom bonded, the presence of other functional groups, steric hindrance, etc. So far, no one has attempted to quantify the energy of these bonds, although there have recently been attempts to employ various thermodynamic data in the study of processes in gas chromatography[5–7]. A single publication on paper chromatography considered the dependence of the magnitude of the R_F values of various aliphatic acids on the energy of the hydrogen bonds found from IR spectroscopy[8].

Attempts to express quantitatively the energy of hydrogen bonds using paper chromatography have already been described[10–18]. All of these works are based on data determined for the energy of the corresponding intramolecular hydrogen bonds by a physico-chemical method, and these data are compared with the magnitude of the shift in the R_F (R_M) values as a result of the formation of internal hydrogen bonds. In this connection, the "equivalent" of the hydrogen bond was introduced (R_E), defined as the energy of a hydrogen bond corresponding to a shift of 0.10 R_M. This equivalent is calculated from the equation

$$R_E = \frac{\Delta E}{\Delta R_M} \cdot 0.1$$

0021-9673/81/0000–0000/$02.50

where ΔE is the energy difference between isomers with and without intra-molecular hydrogen bonds and ΔR_M is the difference in the R_M values.

This paper is a continuation of an earlier study in which we attempted to use R_E values to characterize separation systems, to choose the best stationary phase and to predict the success of the separation. Simultaneously, it was possible to use completely new stationary phases in paper chromatography. This first attempt was carried out using derivatives of anthraquinones, 1- and 2-amino, 1- and 2-hydroxy, 1- and 2-dimethylamino and 1- and 2-methoxyanthraquinone.

EXPERIMENTAL

Survey of the mobile and stationary phases and sample substances used

Eight derivatives of anthraquinone, twenty-seven stationary phases and two types of mobile phase were employed.

The following model substances were used in the chromatographic separation: 1-aminoanthraquinone, 2-aminoanthraquinone, 1-N,N'-dimethylaminoanthraquinone, 2-N,N'-dimethylaminoanthraquinone, 1-hydroxyanthraquinone, 2-hydroxyanthraquinone, 1-methoxyanthraquinone and 2-methoxyanthraquinone.

For cyclohexane–pyridine (25:1) mobile phase, formamide, dimethylformamide, acetamide, acrylamide, ethylene glycol, propylene glycol, 2,3-butylene glycol, glycerine, polyglycol 300, ethanolamine, diethanolamine, monoisopropanolamine and methyl Cellosolve were used as stationary phases. For the ethanol–water mobile phase (in various ratios), methyl palmitate, butyl stearate, dimethylcyclohexyl fumarate, butylheptyl phthalate, dinonyl phthalate, didecyl phthalate, dilauryl phthalate, dioctyl adipate, dioctyl succinate, dioctyl sebacate, methyl myristate, ethyl myristate, butyl salicylate and dodecyl chloroacetate were used as stationary phases.

Chromatography

Chromatography of the anthraquinone derivatives was carried out by the descending technique using Whatman No. 1 paper. The paper was impregnated with the selected stationary phase, dissolved either in ethanol (amides, hydroxyamines, glycols) or in cyclohexane (esters) at concentrations of 20% (w/w), except for formamide and dimethylformamide (30%, w/w).

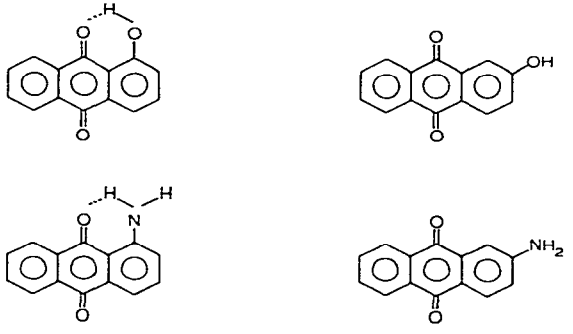
Chromatographic development was carried out at laboratory temperature (18–22°C). Separations in cyclohexane–pyridine took about 4 h and in ethanol–water 15 h or longer (overnight). Dectection was carried out under UV light without spraying.

RESULTS AND DISCUSSION

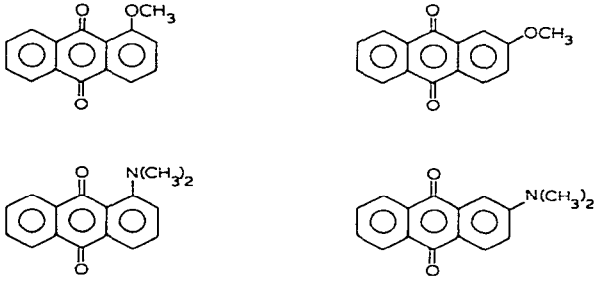
Much new information can be derived from the results given in Tables I–IV. Primarily, it is apparent that the number of possible stationary phases can be increased. For polar stationary phases, various glycols, *e.g.*, butylene glycol, propylene glycol, ethylene glycol and polyglycol 300, amides, in addition to formamide, dimethylformamide and acetamide and also acrylamide and some less volatile amines such as diethanolamine and monoisopropanolamine can be used successfully, and not only for separating anthraquinone derivatives. For non-polar stationary phases, various esters such as dinonyl phthalate, dilauryl phthalate, butylheptyl phthalate,

methyl palmitate, dimethylcyclohexyl fumarate, butyl stearate, dioctyl succinate, dioctyl adipate, dioctyl sebacate, butyl salicylate, ethyl myristate, methyl myristate, didecyl phthalate and dodecyl chloroacetate can be used.

The polar and non-polar stationary phases for separating amino- and hydroxyanthraquinones were chosen in order to demonstrate the effect of intermolecular and intramolecular hydrogen bonds on the course of chromatographic separations and in order to give a quantitative interpretation to this effect. For this purpose, only two mobile phases were chosen, one for polar stationary phases (cyclohexane–pyridine) and one for non-polar stationary phases (aqueous ethanol). It can be seen from the tables that the R_F value for 1-amino- or 1-hydroxyanthraquinone is always greater for a polar stationary phase than for 2-amino- or 2-hydroxyanthraquinone. The results are the opposite for non-polar stationary phases. The reason for this effect has already been discussed[17] and lies primarily in the formation of internal hydrogen bonds with the 1-derivatives:



To confirm this effect, the methyl derivatives were employed in addition to the amino- and hydroxyanthraquinones:



It follows from the R_F values obtained that the internal hydrogen bond O–H ··· O is as strong as if the hydrogen atom on the OH group were replaced by a methyl group and thus the R_F value is almost identical. In the O ··· H–N bond, the remaining hydrogen atom in the NH_2 group still has some energy, so that 1-amino and 2-aminoanthraquinone with a non-polar stationary phase have smaller R_F values than the corresponding methyl derivatives.

With the polar stationary phases, lower R_F values were found for 2-hydroxy-

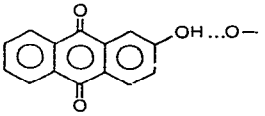
TABLE I

R_F, R_M and ΔR_M VALUES FOR AMINO- AND HYDROXYANTHRAQUINONES AND DIMETHYLAMINO- AND METHOXYANTHRAQUINONES ON A STATIONARY PHASE WITH BOTH DONOR AND ACCEPTOR GROUPS

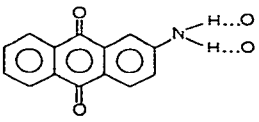
Anthraquinone	*Parameter*	*Stationary phase*				
		Dimethyl-formamide	*Formamide*	*Acetamide*	*Acrylamide*	*Ethylene glycol*
1-Amino-	R_F	0.10	0.34	0.32	0.11	0.44
	R_M	0.95	0.29	0.33	0.91	0.10
	ΔR_M	0.84	1.16	1.36	1.14	1.20
2-Amino-	R_F	0.016	0.034	0.02	0.009	0.049
	R_M	1.79	1.45	1.69	2.04	1.30
1-N,N-Dimethylamino-	R_F	0.41	0.76	0.72	0.37	0.83
	R_M	0.16	−0.50	−0.41	−0.23	−0.69
	ΔR_M	0.30	0.29	0.36	0.32	0.30
2-N,N-Dimethylamino-	R_F	0.26	0.62	0.55	0.22	0.71
	R_M	0.45	−0.21	−0.08	0.55	−0.39
1-Hydroxy-	R_F	0.57	0.84	0.83	0.59	0.89
	R_M	−0.12	−0.72	−0.70	−0.16	−0.83
	ΔR_M	1.37	1.58	1.42	1.86	1.62
2-Hydroxy-	R_F	0.056	0.12	0.16	0.02	0.14
	R_M	1.25	0.86	0.72	1.69	0.79
1-Methoxy-	R_F	0.51	0.81	0.66	0.58	0.80
	R_M	−0.02	−0.63	−0.29	−0.14	−0.60
	ΔR_M	0.04	0.10	0.06	0.085	0.03
2-Methoxy-	R_F	0.49	0.77	0.63	0.53	0.80
	R_M	0.02	−0.53	−0.23	−0.06	−0.63

anthraquinone than for 2-methoxyanthraquinone, indicating that the free OH group can form a hydrogen bond with the stationary phase.

The energies of the hydrogen bonds have been calculated to be approximately O–H · · · O 6 kcal/mol (25 kJ/mol), O · · · H–N 4.5 kcal/mol (18 kJ/mol) and H · · · H–N 3 kcal/mol (12.5 kJ/mol)[9]. Consequently, in a polar stationary phase 1-hydroxyanthraquinone has a higher R_F value than 1-aminoanthraquinone. With stationary non-polar phases, the opposite conditions prevail.



6 kcal/mol (25 kJ/mol)



4.5 kcal/mol (18.8 kJ/mol)
4.5 kcal/mol (18.8 kJ/mol)

Propylene glycol	*Glycerine*	*Polyglycol 300*	*Ethanol-amine*	*Diethanol-amine*	*Monoisopro-panolamine*	*Methyl Cellosolve*	*2,3-Butylene glycol*
0.25	0.67	0.073	0.78	0.56	0.47	–	0.11
0.48	−0.31	1.13	−0.55	−0.10	−0.055	–	0.91
1.08	1.31	0.97	1.05	1.38	1.20	–	1.04
0.027	0.001	0.008	0.24	0.052	0.056	–	0.011
1.56	1.00	2.10	0.50	1.28	1.25	–	1.95
0.67	0.85	0.62	0.90	0.88	0.84	0.68	0.46
−0.31	−0.76	−0.21	−0.95	−0.86	−0.72	−0.33	0.07
0.35	0.18	0.28	0.16	0.10	0.14	0.33	0.30
0.48	0.79	0.46	0.86	0.85	0.78	0.50	0.30
0.04	−0.58	0.07	−0.79	−0.76	−0.58	0.00	0.37
0.81	–	0.36	0.92	0.86	0.80	0.66	0.69
−0.63	–	0.25	−1.09	−0.79	−0.60	−0.29	−0.35
1.49	–	1.73	2.67	2.80	2.30	1.57	1.70
0.12	–	0.01	0.026	0.01	0.019	0.051	0.043
0.86	–	1.98	1.58	2.01	1.70	1.28	1.35
0.73	0.84	0.28	–	0.89	–	0.64	0.61
−0.43	−0.72	0.41	–	−0.91	–	−0.25	−0.19
0.10	0.03	0.00	–	0.05	–	0.07	0.05
0.68	0.83	0.28	–	0.88	–	0.60	0.58
−0.33	−0.69	0.41	–	−0.86	–	−0.18	−0.14

It again follows from these results that an important role in chromatographic separations is played by intermolecular and internal hydrogen bonds, provided that they can be formed in the substances to be separated and in the stationary phase.

It has already been demonstrated[10–18] that the effect of these hydrogen bonds can be quantified and one of the purposes of this work was to extend this information.

The chromatographic equivalent of a hydrogen bond, designated as R_E, was derived in the publications cited. This R_E value is calculated from the equation $R_E = 0.1 \cdot \Delta E/\Delta R_M$, where ΔE is the energy of the hydrogen bond and ΔR_M is difference in the R_M values between the compound with an internal hydrogen bond and an isomer without such a bond. The ΔE value, for example, corresponds to the difference between the heats of sublimation of the particular substances or other data, *e.g.*, the difference in the energies found from the IR spectra. For hydroxy- and aminoanthraquinones, the literature contains data on the heats of sublimation, from which it follows that the energy of the internal hydrogen bond of 1-aminoanthraquinone is 4.8 kcal/mol (20.2 kJ/mol) and of 1-hydroxyanthraquinone 7.8 kcal/mol (32.8 kJ/mol). If these values are taken as a basis for the calculation of R_E for the individual stationary phases, either polar or non-polar, then the order in

TABLE II

R_F, R_M AND ΔR_M VALUES FOR AMINO- AND HYDROXYANTHRAQUINONES AND DIMETHYL AMINO- AND METHOXYANTHRAQUINONES ON STATIONARY PHASES WITH ONLY ACCEPTOR GROUPS

Anthraquinone	*Parameter*	*Stationary phase**				
		Methyl palmitate (50% C_2H_5OH)	*Dimethylcyclohexyl fumarate (40% C_2H_5OH)*	*Butyl stearate (50% C_2H_5OH)*	*Butylheptyl phthalate (50% C_2H_5OH)*	*Dioctyl succinate (66% C_2H_5OH)*
1-Amino-	R_F	0.15	0.26	0.49	0.053	0.15
	R_M	0.77	0.45	0.02	1.27	0.76
	ΔR_M	0.44	0.40	0.25	0.55	0.45
2-Amino-2-	R_F	0.32	0.47	0.63	0.16	0.33
	R_M	0.33	0.05	−0.23	0.72	0.31
1-N,N-Dimethylamino-	R_F	0.20	0.30	0.60	0.061	0.28
	R_M	0.60	0.37	−0.17	1.21	0.41
	ΔR_M	0.15	0.08	0.17	0.24	0.07
2-N,N-Dimethylamino-	R_F	0.15	0.26	0.50	0.035	0.25
	R_M	0.75	0.45	0.00	1.45	0.48
1-Hydroxy-	R_F	0.064	0.13	0.13	0.023	0.088
	R_M	1.17	0.81	0.81	1.62	1.04
	ΔR_M	0.72	0.72	1.06	0.90	0.75
2-Hydroxy-	R_F	0.26	0.45	0.64	0.16	0.34
	R_M	0.45	0.087	−0.25	0.72	0.29
1-Methoxy-	R_F	0.058	0.13	0.26	0.019	0.09
	R_M	1.24	0.81	0.45	1.71	1.02
	ΔR_M	0.15	0.21	0.20	0.24	0.30
2-Methoxy-	R_F	0.079	0.20	0.36	0.032	0.16
	R_M	1.09	0.60	0.25	1.47	0.72

which the R_E values change can be found. More careful comparison of the individual R_E values has shown that their magnitude depends directly on the sum of the energies of all possible hydrogen bonds in the stationary phase, of both donor and acceptor functional groups.

The following review gives a summary of all possible intermolecular hydrogen bonds for polar and non-polar stationary phases.

If the R_E values are plotted as a function of the energy of possible hydrogen bonds, a set of points is obtained (Fig. 1), through which a straight line can be drawn,

Dioctyl adipate (50% C_2H_5OH)	*Dioctyl sebacate (66% C_2H_5OH)*	*Dodecyl chloroacetate (40% C_2H_5OH)*	*Methyl myristate (66% C_2H_5OH)*	*Ethyl myristate (66% C_2H_5OH)*	*Dinonyl phthalate (80% C_2H_5OH)*	*Didecyl phthalate (70% C_2H_5OH)*	*Dilauryl phthalate (80% C_2H_5OH)*	*Butyl salicylate (70% C_2H_5OH)*	*SiO_2 (30% toluene–dimethylformamide in C_2H_5OH)*
0.09	0.22	0.20	0.10	0.13	0.27	0.33	0.27	0.41	0.15
1.02	0.55	0.60	0.95	0.83	0.43	0.31	0.43	0.16	0.76
0.54	0.38	0.46	0.42	0.35	0.43	0.38	0.31	0.53	−0.87
0.25	0.40	0.42	0.23	0.25	0.50	0.54	0.43	0.70	0.023
0.48	0.17	0.14	0.53	0.48	0.00	−0.07	0.12	−0.37	1.63
0.15	0.31	0.18	0.12	0.16	0.32	0.37	0.25	0.35	0.059
0.76	0.35	0.66	0.86	0.72	0.33	0.23	0.48	0.27	1.23
0.22	0.08	0.13	0.13	0.11	0.15	0.22	0.10	0.02	0.21
0.095	0.27	0.14	0.093	0.13	0.25	0.26	0.21	0.34	0.09
0.98	0.43	0.79	0.99	0.83	0.48	0.45	0.58	0.29	1.02
0.032	0.13	0.068	0.051	0.066	0.068	0.17	0.10	0.095	0.38
1.48	0.82	1.16	1.28	1.18	1.12	0.69	0.95	0.97	0.21
0.85	0.70	1.06	0.75	0.63	0.76	0.79	0.78	0.95	−1.61
0.19	0.43	0.44	0.23	0.22	0.30	0.56	0.40	0.49	0.015
0.63	0.12	0.10	0.53	0.55	0.37	−0.10	0.17	0.02	1.82
0.046	0.10	0.067	0.056	0.069	0.072	0.14	0.12	0.10	0.37
1.32	0.95	1.17	1.24	1.16	1.10	0.79	0.86	0.95	0.23
0.15	0.26	0.22	0.14	0.21	0.28	0.21	0.17	0.16	−0.22
0.066	0.17	0.10	0.079	0.10	0.13	0.21	0.14	0.14	0.26
0.17	0.69	0.95	1.10	0.95	0.82	0.58	0.79	0.79	0.45

* (% C_2H_5OH) indicates the mobile phase used for the particular stationary phase. The R_F values are averages from several chromatograms.

expressed by the following equations (for the polar stationary phase):

$$R_E = -0.01\ X + 0.63$$

for the phase with both donor and acceptor groups, and

$$R_E = -0.01\ X + 1.25$$

for the phase with acceptor groups, where X is the overall energy of the hydrogen bonds to both donor and acceptor groups (Table V).

TABLE III

FOUND AND CALCULATED R_E VALUES FOR STATIONARY PHASE WITH DONOR AND ACCEPTOR GROUPS

Mobile phase: cyclohexane–pyridine (25:1). R_E (exp.) is the R_E value obtained from the experimental ΔR_M values using the relationship $R_E = 0.1\ \Delta E/\Delta R_M$. The column designated NH_2 contains R_E (exp.) values calculated from data obtained from chromatography of aminoanthraquinones, and the column designated OH contains R_E (exp.) values obtained for hydroxyanthraquinones. The R_E (calc.) values were obtained from the equation $R_E = f(x)$ found by linear regression.

Stationary phase	R_E *(exp.)*			R_E *(calc.)*	ΔR_E
	NH_2	*OH*	*Average*		
Dimethylformamide	0.57	0.57	0.57	0.58	+0.01
Formamide	0.41	0.49	0.45	0.50	+0.05
Acetamide	0.35	0.55	0.45	0.50	+0.05
Acrylamide	0.42	0.42	0.42	0.48	+0.06
Propylene glycol	0.44	0.52	0.48	0.43	−0.05
2,3-Butylene glycol	0.46	0.46	0.46	0.43	−0.03
Ethylene glycol	0.40	0.48	0.44	0.43	−0.01
Polyglycol 300	0.49	0.46	0.48	0.43	−0.05
Methyl Cellosolve	–	0.48	0.48	0.48	0.00
Glycerine	0.36	–	0.36	0.34	−0.02
Ethanolamine	0.46	0.32	0.39	0.45	+0.06
Diethanolamine	0.35	0.29	0.32	0.40	+0.08
Monoisopropanolamine	0.40	0.34	0.37	0.45	+0.08

TABLE IV

FOUND AND CALCULATED R_E VALUES FOR STATIONARY PHASE WITH ONLY ACCEPTOR GROUPS

Mobile phase: ethanol–water (in various ratios; see Table II). R_E values obtained as in Table III.

Stationary phase	R_E *(exp.)*			R_E *(calc.)*	ΔR_E
	NH_2	*OH*	*Average*		
Dinonyl phthalate	1.11	1.03	1.07	1.05	−0.02
Didecyl phthalate	1.26	1.00	1.13	1.05	−0.08
Dilauryl phthalate	1.55	1.00	1.27	1.05	−0.22
Butylheptyl phthalate	0.88	0.87	0.88	1.05	+0.17
Butyl salicylate	0.91	0.81	0.86	1.05	+0.19
Dioctyl succinate	1.07	1.04	1.06	1.05	−0.01
Dioctyl adipate	0.89	0.92	0.91	1.05	+0.14
Dioctyl sebacate	1.26	1.12	1.19	1.09	−0.14
Methyl palmitate	1.09	1.08	1.09	1.17	+0.08
Butyl stearate	1.92	0.74	1.33	1.17	−0.16
Methyl myristate	1.14	1.04	1.09	1.17	+0.04
Ethyl myristate	1.36	1.24	1.30	1.17	−0.13
Dimethylcyclohexyl fumarate	1.20	1.03	1.14	1.05	−0.09
Dodecyl chloroacetate	1.04	0.74	0.89	1.17	+0.16

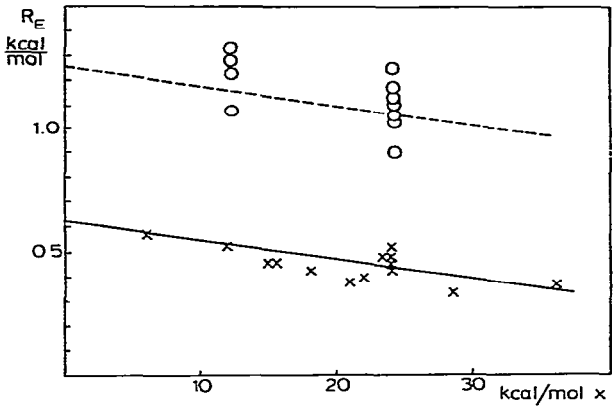


Fig. 1. Dependence of R_E values on the energy of the hydrogen bond. ×, Stationary phases with both donor and acceptor groups; O, stationary phase with only acceptor groups (except for butyl salicylate).

A similar procedure was employed for the other stationary phases (Table VI).

It follows from this dependence that the greatest differences in the R_F values for substances with and without intramolecular hydrogen bonds will be found for stationary phases for which the sum of the energies of all possible hydrogen bonds is greatest.

For example, ΔR_M for 1-amino- and 2-aminoanthraquinone on dimethylformamide (where all possible hydrogen bonds have an energy of about 6 kcal/mol) is 0.84, whereas on diethanolamine (where all possible hydrogen bonds have an energy of 28.5 kcal/mol) ΔR_M is 1.38. Similarly, for 1- and 2-hydroxyanthraquinone ΔR_M = 1.37 and 2.80, respectively.

It therefore follows that the smaller the R_E value for the given system, the better is the separation of substances with donor functional groups. Thus the R_E values can be used for the quantitative evaluation of the separation of particular substances.

A further group of compounds studied were the esters of mono- and dibasic acids. As these stationary phases have no donor group, their separation ability is poorer.

If the linear equation given for the polar phases were valid, but with a slope of opposite sign, then it would follow for those stationary phases with an overall energy of possible hydrogen bonds of 12 kcal/mol that R_E = 0.89 and for those with an energy of 24 kcal/mol that R_E = 1.13. Consequently, this is in reasonable agreement, although it should be taken into consideration that the energy of the hydrogen bonds is also affected by the size of the alkyl groups in these stationary phases. It was calculated for the second group of stationary phases that the energy of the hydrogen bonds is 24 or 12 kcal/mol, depending on the number of carboxyl groups. It is necessary, however, to recall that the actual energy will differ as a result of the different sizes of the alkyl residues in the stationary phases, which was not taken into consideration in the rough calculation.

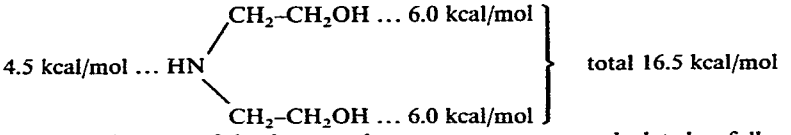
In this entire system, only one stationary phase has a donor group, butyl salicylate, which has an R_E value of 0.77.

If the R_E values are calculated and compared with the values found, then it

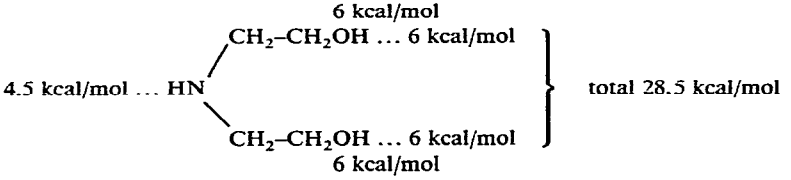
TABLE V

OVERALL ENERGIES OF POSSIBLE HYDROGEN BONDS

The energy of the donor groups, *e.g.* for diethanolamine, was calculated in the following manner:



The overall energy of the donor and acceptor groups was calculated as follows:



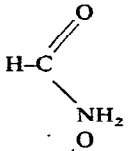
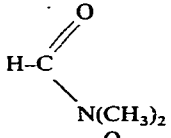
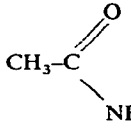
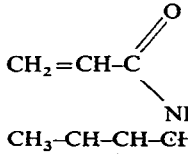
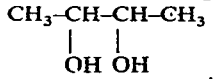
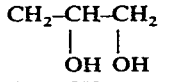
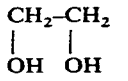
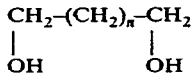
Stationary phase	*Energy of donor groups (kcal/mol)*	*Energy of donor and acceptor groups (kcal/mol)*
	9.0	15.0
	0.0	6.0
	9.0	15.0
	9.0	18.0
	12.0	24.0
	12.0	24.0
	12.0	24.0
	12.0	24.0

TABLE V *(continued)*

Stationary phase	*Energy of donor groups (kcal/mol)*	*Energy of donor and acceptor groups (kcal/mol)*
CH_2–CH_2 | | OH OCH_3	6.0	18.0
CH_2–CH–CH_2 | | | OH OH OH	18.0	36.0
CH_2–CH_2 | | NH_2 OH	15.0	21.0
CH_2–CH_2–NH–CH_2–CH_2 | | OH OH	16.5	28.5
CH_2–$CH(CH_3)$ | | NH_2 OH	15.0	21.0

follows that the differences are not large and, if they are, they can be attributed to the rough estimate of the bonding energy and the actual chromatographic evaluation, especially where the spots of the separated substances have R_F values close to the start or the front, where small differences in the R_F value have a large effect on the R_M value used for the overall calculation.

The R_L value for stationary phases with only acceptor groups is shifted by 0.63 kcal/mol (2.65 kJ/mol) compared with the R_E value for the phase with both donor and acceptor groups, *i.e.*, it is about twice as large for the same energy. This was found earlier and no satisfactory explanation has been found for whether this is accidental and the R_L value changes continuously or whether a real step change occurs on a change in the chromatographic separation conditions. Only further experiments can provide an explanation.

If, on the other hand, the R_E values are used to calculate the energy differences between individual compounds, then these values can be used to explain the chromatographic behaviour of the individual substances found experimentally. If, for example, the R_E values are used to calculate the energy differences of the hydrogen bonds between 2-hydroxyanthraquinone and 3-methoxyanthraquinone, an average difference of 7.2 kcal/mol (30 kJ/mol) is found for a polar stationary phase, corresponding to a single OH···O bond.

If the same calculations are carried out for 1-aminoanthraquinone and 1-dimethyl aminoanthraquinone, a difference of 3.6 kcal/mol (15.1 kJ/mole) is found. Consequently, the NH_2 group in 1-aminoanthraquinone is bonded through a single hydrogen bond to the oxygen of the anthraquinone skeleton and through another hydrogen bond to the stationary phase:

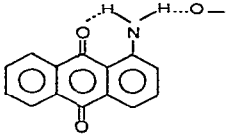
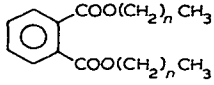
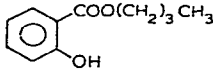
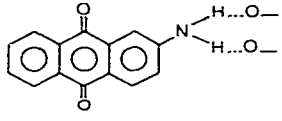


TABLE VI

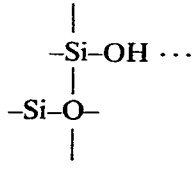
OVERALL ENERGIES OF POSSIBLE HYDROGEN BONDS FOR STATIONARY PHASES WITH ONLY ACCEPTOR GROUPS

Stationary phase	*Energy of possible hydrogen bonds (kcal/mol)*
 (dialkyl phthalate; $n = 1, 2 \ldots$)	24.0
 (butyl salicylate)	24.0
$CH_3(CH_2)_7OOCCH_2CH_2COO(CH_2)_7CH_3$ (dioctyl succinate)	24.0
$CH_3(CH_2)_7OOC(CH_2)_4COO(CH_2)_7CH_3$ (dioctyl adipate)	24.0
$CH_3(CH_2)_{14}COOCH_3$ (methyl palmitate)	12.0
$CH_3(CH_2)_{16}COO(CH_2)_3CH_3$ (butyl stearate)	12.0
$CH_3(CH_2)_{12}COOCH_3$ (methyl myristate)	12.0
$ClCH_2COO(CH_2)_{11}CH_3$ (dodecyl chloroacetate)	12.0
$CH_3OOC-C(C_6H_{11})=CH-COOCH_3$ (dimethylcyclohexyl fumarate)	24.0
$CH_3(CH_2)_7OOC(CH_2)_8COO(CH_2)_7CH_3$ (dioctyl sebacate)	24.0

Similarly, a difference of $\Delta R_M \approx 8$ kcal/mol is found for 2-aminoanthraquinone and 2-dimethylanthraquinone; this corresponds to the bonds shown below:



Apart from the chosen stationary phase, calculation of R_E was carried out for silica gel as a stationary phase with toluene–30% dimethylformamide in ethanol (10:2) as the mobile phase. Using the relationship given above, a value of $R_E = 0.43$ kcal/mol was calculated assuming that silica gel has both donor and acceptor groups:



A value of $R_E = 1.13$ was found (for both amino- and hydroxyanthraquinones). This behaviour corresponds to the presence of only acceptor groups with an overall energy of about 12 kcal/mol. This is also confirmed by the smaller differences in the R_F values for the 1- and 2-isomers. This phenomenon will be studied in greater detail.

The work of Chasar[4] should be mentioned here. He studied the chromatography of 1,8-dihydroxyanthraquinone and its methoxy derivatives on silica gel with a chloroform mobile phase and found the following R_F values: 1,8-dihydroxyanthraquinone, 0.57; 1-hydroxy-8-methoxyanthraquinone, 0.41; and 1,8-dimethoxyanthraquinone, 0.86. A value of $R_E = 1.12$ for a single hydrogen bond follows from the R_M values for this system. The difference (ΔR_M) for two hydrogen bonds indicates that the energy of the second bond is less than half as great. This is understandable, as the formation of the first bond decreases the electronegativity of the oxygen.

Schulz and Herrman[19] chromatographed some hydroxybenzoic acids on silica gel with dichloromethane–toluene–formic acid (5:4:1) as the mobile phase. If a hydrogen bond energy of 5 kcal/mol (21 kJ/mol)[9] is calculated for salicylic acid, it follows that $R_E = 1.16$.

Comparison of the R_L values for aminoanthraquinones[16] is also interesting, yielding $R_E = 0.65$; chromatography was carried out on a poured layer of aluminium oxide.

So far it is not possible to decide from these data whether the given relationships are generally valid for the calculation of R_L values. It is apparent that further measurements should be carried out with other types of substances in order to decide whether the relationship is generally valid or is valid in a given form only for a particular group of substances. A difficulty is encountered in that the literature generally contains thermodynamic data only for simple substances or for a single substance in a given series which is separated chromatographically.

Nonetheless, the importance of hydrogen bonds for chromatographic separations and the quantitative expression of their energies has been verified and contributes to the general clarification of the chromatographic process taking place and permits the prediction of the behaviour of substances during the separation, choice of a suitable stationary phase and, hopefully in the future, expression of the difference in the energies of intermolecular bonds.

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