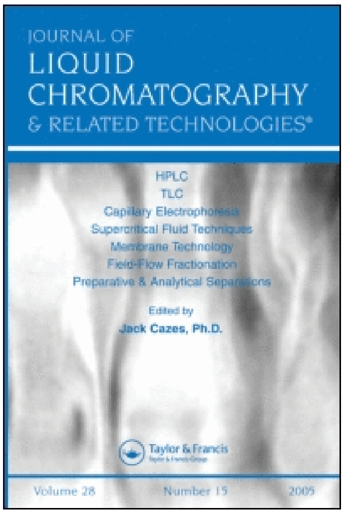

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Retention Behavior of Some Thiophosphorylglycinamide Fungicides in Adsorption and Reversed-Phase Thin-Layer Chromatography

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Retention Behavior of Some Thiophosphorylglycinamide Fungicides in Adsorption and Reversed-Phase Thin-Layer Chromatography

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Abstract: The free energy of adsorption, the surface area of adsorption on silica and alumina surfaces, and the molecular lipophilicity of 37 thiophosphorylglycinamide (TPGA) derivatives was determined by thin layer chromatography using silica and alumina supports and silica support impregnated with paraffin oil. The relationships between the physicochemical characteristics of analytes were elucidated by stepwise regression analysis. It was found that the physicochemical parameters showed high variety between the derivatives, and they depended markedly on the silica and alumina surfaces. In the majority of cases, no linear correlation was found between the parameters.

The free energy of adsorption and the surface area of adsorption on the silica surface showed highly significant correlation, indicating that the energy of adsorption per unit surface is similar for each of the TPGA derivatives and the molecular substructures involved in the adsorption are probably chemically similar.

Keywords: Thiophosphorylglycinamide derivatives, Free energy of adsorption, Adsorbed surface area, Liphophilicity

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INTRODUCTION

In the last decades, various thin-layer chromatography (TLC) techniques have been extensively applied for the separation and semi-quantitative determination of a wide variety of organic and inorganic compounds. The main advantages of TLC methods are the possibility of parallel determination, the speed of the analysis, and the low requirement of complicated instrumentation. Earlier results in this interesting field of chromatographic research have been previously compiled and discussed in detail.[1]

TLC has been recently applied for the determination of arginine in dietary supplements,[2] the separation of model peptides in normal phase TLC,[3] the analysis of gangliosides[4] and curcumin,[5] the separation of opiate alkaloids,[6] and for the determination of lamotrogine in serum,[7] pesticides in honey,[8] and anthraquinone derivatives in *Rheum emodi*.[9]

However, TLC techniques can be employed, not only for the separation of analytes, but also for the determination of various molecular parameters, such as the free energy of adsorption, surface area of adsorbed molecules, the lipophilicity of bioactive compounds,[10] relative strength of interaction between two different molecular species,[11] etc.

Linear and multiple linear regression analyses have been frequently applied in the evaluation of retention data in various chromatographic techniques for the assessment of the relationship between one dependent and one or more independent variables. Because of their simplicity and good predictive power, they have been successfully applied for the study of the molecular basis of separation,[12] the classification of new stationary phases,[13] quantitative structure-retention relationship computations in both high performance liquid chromatography (HPLC)[14] and gas chromatography (GC),[15,16] and the elucidation of the correlation between retention characteristics and biological activity.[17]

Stepwise regression analysis (SRA) is an up-to-date version of multivariate linear regression analysis. In the traditional multivariate regression analysis, the presence of independent variables that exert no significant influence on the dependent variable decreases the significance level of the independent variables that significantly influence the dependent variable. SRA overcomes this difficulty by automatically eliminating from the selected equation the insignificant independent variables, thereby enhancing the information power of the computation.[18]

A new type of thiophosphorylglycineamide (TPGA) derivatives with broad spectrum of acaricidal and fungicidal activity was developed at North Hungarian Chemical Works (NHCW, Sajóbábony, Hungary). Their biological activity, using principal component analysis and the spectral mapping technique, has been studied in detail.[19]

The objectives of the investigations were the determination of the free energy of adsorption, surface area of adsorbed TPGA derivatives on both silica and alumina surfaces, the assessment of their lipophilicities using reversed-phase TLC, and the elucidation of the relationships between the molecular physicochemical parameters measured by TLC. The results may promote a better understanding of their mode of action and may help in the prediction of their behavior in adsorptive and hydrophobic environmental conditions.

EXPERIMENTAL

Materials

TPGA derivatives were the gift of NHCW and were used as received. Their chemical structures are compiled in Table 1. DC-Alufolien silica gel $60F_{254}$ and DC-Alufolien aluminium oxide $60F_{254}$ (both 20×20 cm; layer thickness, 0.2 mm) were purchased from Merck AG (Darmstadt, Germany) and were not pretreated, neither by prewashing or by activation at elevated temperature. The solvents, *n*-pentane, *n*-hexane, benzene, and acetone, of analytical grade, were purchased from Sigma-Aldrich Kft (Budapest, Hungary). *n*-Pentane, benzene, and acetone were distilled prior to use. Paraffin oil of pharmaceutical quality was purchaesd in a local pharmacy.

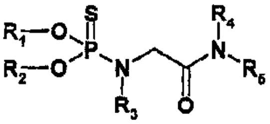
Determination of the Free Energy of Adsorption (S_o) and Adsorbed Surface Area (A_s) in Normal-Phase TLC

TPGA derivatives were separately dissolved in ethanol at a concentration of 1% (w/v), and 2 μL of the solution was spotted onto the plates with a micropipette (Camag, Switzerland). The compositions of ternary mobile phases and the calculated solvent strengths (ε^o) are listed in Table 2. Solvent strengths of ternary mobile phases were calculated according to the linear additivity rule. We are well aware that this method of calculation does not take into consideration the interactions between the different molecules of the mobile phase that can modify the solvent strength. Unfortunately, data on the solvent strengths of these ternary systems are not presently available in the literature.

Developments were carried out in sandwich chambers of $21\,\text{cm} \times 20\,\text{cm} \times 0.3\,\text{cm}$ at 4–5°C. The application of low temperature was motivated by the high volatility of *n*-pentane which can uncontrollably modify the composition of the mobile phase and its solvent strength. The development distance was about 100–120 mm in each instance. After development, the plates were dried at room temperature, and the spots were detected with iodine vapor. The spot center was determined visually. The R_M value used for further computations was calculated for each of the TPGA derivatives in each adsorption TLC system by

$$R_M = \log(1/R_F - 1) \qquad (1)$$

Table 1. Chemical structures of thiophosphorylglycineamide derivatives general structure



No.	R_1 and R_2	R_3	R_4	R_5
1	Methyl	*n*-Propyl	Benzyl	H
2	Methyl	Ethyl	*i*-Butyl	*i*-Butyl
3	Methyl	*n*-Propyl	*i*-Butyl	*i*-Butyl
4	Ethyl	Ethyl	Ethoxypropyl	H
5	Ethyl	Ethyl	Cyclohexyl	H
6	Ethyl	Ethyl	Benzyl	H
7	Ethyl	Ethyl	2-Chlorophenyl	H
8	Ethyl	Ethyl	4-Chlorophenyl	H
9	Ethyl	Ethyl	2,6-Dimethylphenyl	H
10	Ethyl	Ethyl	2-Methyl-6-ethylphenyl	H
11	Ethyl	Ethyl	3-Trifluoromethylphenyl	H
12	Ethyl	Ethyl	3-Trifluoromethyl -4-chlorophenyl	H
13	Ethyl	*n*-Propyl	Ethoxypropyl	H
14	Ethyl	Allyl	Ethoxypropyl	H
15	Ethyl	*n*-Propyl	Morphonyl	H
16	Ethyl	*n*-Propyl	Piperidyl	H
17	Ethyl	Allyl	Allyl	H
18	Ethyl	butyl	Benzyl	H
19	Ethyl	Ethyl	*n*-Propyl	*n*-Propyl
20	Ethyl	Ethyl	*i*-Propyl	*i*-Propyl
21	Ethyl	Ethyl	*i*-Butyl	*i*-Butyl
22	Ethyl	Ethyl	Cyclohexyl	Methyl
23	Ethyl	Ethyl	Cyclohexyl	Ethyl
24	Ethyl	Ethyl	Phenyl	*i*-Propyl
25	Ethyl	Ethyl	*2*-Methyl-6-ethylphenyl	Methoxymethyl
26	Ethyl	*n*-Propyl	*n*-Propyl	*n*-Propyl
27	Ethyl	*n*-Propyl	*i*-Propyl	*i*-Propyl
28	Ethyl	*n*-Propyl	*i*-Butyl	*i*-Butyl
29	Ethyl	*n*-Propyl	Cyclohexyl	Ethyl
30	Ethyl	*n*-Propyl	Phenyl	*i*-Propyl
31	Ethyl	Allyl	*n*-Propyl	*n*-Propyl
32	Ethyl	Allyl	Phenyl	*i*-Propyl
33	Ethyl	*i*-Propyl	*i*-Propyl	*i*-Propyl
34	Ethyl	*n*-Butyl	*n*-Propyl	*n*-Propyl
35	Ethyl	*i*-Butyl	*n*-Propyl	*n*-Propyl
36	Ethyl	Ethoxypropyl	*n*-Propyl	*n*-Propyl
37	Ethyl	Ethoxypropyl	Phenyl	*i*-Propyl

Table 2. Composition of ternary mobile phases used for adsorption TLC measurements (vol. %) (ϵ° = calculated solvent strength)

Solvents		*n*-Pentane		Benzene		Acetone	
Adsorbents		Silica	Alumina	Silica	Alumina	Silica	Alumina
No.	ϵ°						
1	0	100	100	0	0	0	0
2	0.10	99.0	95.25	1.0	4.0	0	0.75
3	0.15	74.0	90.25	26.0	8.0	0	1.75
4	0.20	48.0	83.0	52.0	14.0	0	3.0
5	0.25	49.25	71.0	50.0	24.5	0.75	4.5
6	0.30	48.0	52.0	48.5	41.5	3.5	6.5
7	0.35	45.0	40.5	45.5	47.0	9.5	12.5
8	0.40	38.0	36.0	37.5	41.0	24.5	23.0
9	0.45	17.5	29.0	17.5	32.0	65.0	39.0
10	0.50	0	19.5	0	20.0	0	60.5
11	0.55	0	4.0	0	3.5	0	92.5

Both adsorption and reversed-phase measurements were carried out in triplicate; the data were omitted from the following calculations when the relative standard deviation between the parallel depermination was higher than 5%.

The free energy of adsorption and the surface area of adsorbed TPGA derivatives were calculated from the relationships between the retention parameters and mobile phase characteristics.

Determination of the Lipophilicity in Reversed-Phase TLC

Silica plates were impregnated by overnight predevelopment in *n*-hexane-paraffin oil (95 : 5, v/v). Mobile phases were mixtures of acetone-water with the concentration of organic modifier varying in steps of 5 vol.%. Other chromatographic parameters were the same as described in the previous section. In order to increase the reliability of the calculation of lipophilicity, the R_M values were extrapolated to zero concentration of organic modifier by

$$R_M = R_{M0} + b.C \tag{2}$$

where R_M is the R_M value of an analyte measured at a given concentration of organic modifier in the mobile phase, R_{M0} is the R_M value extrapolated to water (best estimation of molecular liphilicity), and b is the change of R_M value caused by a 1% change of organic modifier in the mobile phase (related to the hydropobic surface area of the molecule). Calculation was carried out separately for each TPGA derivative.

Calculation of the Relationships Between Adsorptive and Reversed-Phase TLC Systems

The relationships between the various physicochemical parameters of TPGA derivatives measured by TLC techniques was elucidated by stepwise regression analysis (SRA). Computation was carried out by each parameter being separately dependent and independent variable. The number of accepted independent variables was not limited, and the limit of acceptance was set to the 95% significance level. Software for SRA was purchased from Compudrug Ltd. (Budapest, Hungary). The significant differences between the free energy of adsorption and adsorbed surface area determined on silica and alumina stationary phases were calculated by using the paired-means method and the calculated *t*-values were compared with the tabulated ones.

RESULTS AND DISCUSSION

Each analyte displayed a regular spot shape in each TLC system; deformed spots decreasing the reliability of the analysis were not observed. TPGA derivatives showed typical retention behavior: R_M values decreased linearly with increasing solvent strength of the mobile phases in both adsorption and reversed-phase TLC.

The free energy of adsorption (S_o), adsorbed surface area (A_s), and lipophilicity (R_{M0}) of TPGA derivatives are compiled in Table 3. It can be established, from the data in Table 3, that the physicochemical parameters of analytes measured by adsorption and reversed-phase TLC show high variation. This fact indicates that reversed-phase TLC and adsorption TLC carried out on both silica and alumina stationary phase can be successfully used for the separation of TPGA derivatives. Moreover, it can be concluded that the type of substituents exerts a considerable impact on the lipophilicity, free energy of adsorption, and adsorbed surface area of the derivatives.

Stepwise regression analysis found only two significant relationships between the physicochemical parameters. The lipophilicities of analytes were significantly correlated by the free energy of adsorption on alumina surface (n = 37):

$$R_{M0} = 4.23 - 0.285.S^{\circ}(\text{alumina}) \tag{3}$$

$$r = 0.3485 \quad r^2 = 0.1215 \quad F_{(\text{calc.})} = 4.84\ (3.28\%)$$

where n is the number of data points, r is the calculated coefficient of correlation, r^2 is the coefficient of determination, indicating the ratio of variance of dependent variables explained by the independent variable selected by SRA, and $F_{(\text{calc.})}$ is an indicator of the fitness of the equation to the experimental data.

Although the correlation is significant and proves that the retention order of analytes is opposite in adsorption and reversed-phase TLC [negative

Table 3. Lipophilicity (R_{M0}), free energy of adsorption (S°), and adsorbed surface area (A_s) of TPGA derivatives determined by adsorption and reversed-phase thin-layer chromatography (numbers refer to TPGA derivatives in Table 1)

		Adsorbent alumina		Silica	
No.	R_{M0}	S°	A_s	S°	A_s
1	1.980	6.819	8.141	7.637	15.005
2	2.331	5.918	8.179	7.443	15.824
3	2.911	6.025	9.851	7.044	15.026
4	3.191	7.100	8.781	6.710	11.105
5	2.195	7.216	10.254	12.092	29.216
6	1.678	6.799	7.616	7.048	13.218
7	2.684	6.216	9.888	5.580	11.442
8	3.651	6.590	8.603	6.948	15.159
9	2.043	6.535	7.694	7.776	15.786
10	2.223	6.982	9.980	7.798	15.917
11	2.972	6.301	8.562	6.917	15.388
12	3.327	5.844	10.240	6.422	15.291
13	1.724	6.734	7.589	7.813	14.406
14	1.561	7.378	10.243	7.582	13.847
15	1.276	7.213	9.677	8.089	15.746
16	2.093	6.575	9.875	7.844	16.403
17	1.930	6.402	7.131	9.334	21.247
18	2.946	7.545	12.197	8.043	16.891
19	3.668	5.901	7.789	7.053	14.557
20	2.181	5.731	8.122	6.689	13.897
21	2.594	5.742	8.915	7.448	16.658
22	2.862	6.572	10.941	8.187	17.941
23	2.565	6.041	8.507	6.693	13.811
24	2.555	6.182	10.202	7.274	16.063
25	3.368	6.168	10.734	7.832	17.910
26	2.351	5.873	8.692	7.199	15.528
27	2.555	5.729	8.988	6.649	14.213
28	3.327	5.930	11.748	9.159	22.304
29	3.051	5.599	9.182	6.983	15.768
30	3.517	5.983	8.930	6.696	15.273
31	3.831	5.905	8.851	6.601	13.606
32	2.174	6.216	10.361	8.815	20.247
33	3.551	3.162	10.555	8.649	20.171
34	2.292	3.338	12.087	6.993	15.044
35	3.032	5.803	9.913	7.134	16.237
36	2.375	6.225	8.522	8.048	16.723
37	2.817	6.176	8.390	7.927	16.473

retention coefficient of S°(alumina)], the ratio of variance explained is fairly low (12.15%).

As was expected, the adsorption parameters (free energy of adsorption and adsorption surface area) measured on alumina did not correlate with the other physicochemical characteristics. This finding indicates that the retention behaviors of alumina and silica stationary phases show great differences, suggesting again that the retention order of TPGA derivatives and the selectivity of separation may be markedly different on the two adsorptive stationary phases. The fact that the free energy of adsorption and the adsorbed surface area are not correlated suggests that the molecular substructures involved in the adsorption differ considerably among the analytes and the free energy of adsorption does not depend on the adsorbed surface area.

A highly significant realationship was found between the free energy of adsorption and the adsorptive surface area of analytes on the silica surface:

$$S_o = 2.43 + 0.317.A_{s(silica)} \qquad (4)$$

$$r = 0.9297 \qquad r^2 = 0.8644 \qquad F_{(calc.)} = 223.08$$

The correlation suggests that, in contrast to alumina, in the case of silica stationary phase, the same molecular substructures are responsible for the adsorbed surface, and the energy of adsorption markedly depends upon the adsorbed surface areas of analytes.

The calculated *t*-values, indicating the significance level of differences between the paired-means, were $t_{calc.} = 6.73$ and $t_{calc.} = 12.86$ for the free energy of adsorption and adsorbed surface area on silica and alumina stationary phases. The calculations proved that TPGA derivatives bind significantly more strongly to silica than to alumina and the adsorbed surface area is also significantly higher on silica than on alumina.

CONCLUSIONS

It can be concluded, from the data, that both adsorptive and reversed-phase thin layer chromatography can be employed for the determination of various molecular parameters of thiophosphorylglycinamide derivatives. The free energy of adsorption and the adsorption surface area deviate considerably, indicating that the TLC techniques applied can be used for their separation by both adsorption and reversed-TLC.

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