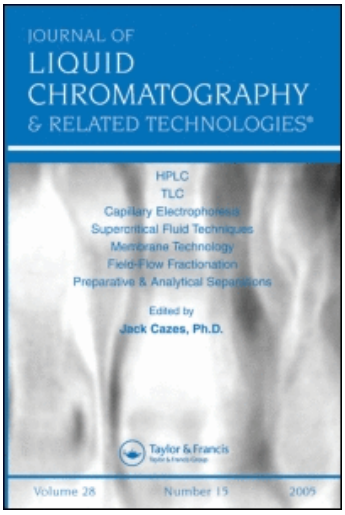

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STUDY OF THE LIPOPHILIC CHARACTER OF XANTHINE AND ADENOSINE DERIVATIVES. II. RELATIONSHIPS BETWEEN LOG K', R_M AND LOG P VALUES

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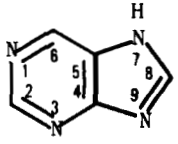
ABSTRACT

The log k' values of a series of xanthine and adenosine derivatives were measured by means of a reversed-phase HPLC. The HPLC data were shown to be well correlated with previously reported R_M and R_{MC18} values. The equations describing the relationships log k'/R_M and log k'/R_{MC18} allowed the calculation of the log k' values of some compounds, which were not tested in the HPLC system. Since the relationship log k'/log P is very close to the previously described relationships R_M /log P and R_{MC18} /log P one can conclude that reversed-phase TLC and HPLC are very similar in describing the lipophilicity of the compounds.

INTRODUCTION

In recent years much effort has been devoted to the study of the pharmacological properties and mecchanisms of action of the xanthine and adenosine derivatives (1,2,3).



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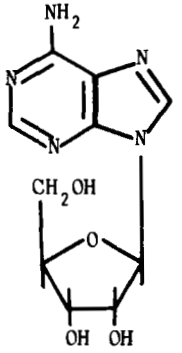

(II)

Purine (I) and its derivatives xanthine (2,6-dioxopurine), adenine (6-aminopurine) and guanine (2-amino-6-oxopurine) are the parent compounds of several classes of very important biologically active chemicals. The purine and guanine derivatives, such as 6-mercaptopurine and 6-thioguanine, represent a group of potential anticancer agents(1). Allopurinol, which is an isomer of 6-oxopurine (hypoxanthine), decreases uric acid production inhibiting xanthine oxidase (1). The pharmacological actions of the classical natural methylxanthines such as caffeine, theobromine and theophylline are well known (1). Recently, the role of xanthines as antagonists of the physiological effects of adenosine (II) emerged as an explanation of their mechanism of action (2,3).

Quantitative structure-activity relationships studies revealed the influence of the lipophilic character on several biological activities of purine derivatives (4). However in most of the studies the lipophilic character was expressed by means of the calculated Hansch's π values and only a few experimental log P or log k' values were measured and reported (5,6,7,8). In a previous paper (4) the lipophilic character of a number of xanthine and adenosine derivatives was expressed by means of the R_M values, obtained from reversed-phase TLC and HPTLC. The R_M values were compared with calculated or experimental log P values. As part of an ongoing programme of work related to a QSAR study dealing with adenosine receptors binding, the purpose of the present paper was a further contribution to the study of the lipophilicity of these compounds by means of a reversed-phase HPLC technique. It is also intended that this work will show the mutual usefulness of both R_M and log k' values in checking the reliability of calculated log P values.

MATERIALS AND METHODS

Chemicals. Xanthine and adenosine derivatives 8-42 had been purchased from RBI (Natick, Mass., U.S.A.); compounds 1-7 and 43 had been obtained from Sigma (St. Louis, Mo., U.S.A.). However in

the present work the log k' values were determined for a series of compounds somewhat smaller of that used previously for the study of the R_M, $R_{M\,C18}$ and log P values. All other chemicals and solvents were of analytical reagent or HPLC grade. In the following we shall refer to any purine derivative as xanthines, and to any nucleoside as adenosines or guanosines.

Determination of log k' values by means of reversed-phase HPLC. Chromatography was performed an a Waters 6000 A chromatograph using a μBondapack C_{18} column (300x3.9 mm I.D.) (Waters, Milford, MA, U.S.A.) packed with Silica Gel (particle size 10 μm) with a C_{18} chemically bonded non-polar stationary phase. A UV detector (Waters Model 480) at 273 nm and Hamilton 802 chromatographic syringes (25 μl) were also used. The test compounds were separated at pH 7.0 using water or methanol-water mixtures as the mobile phase at a flow-rate of 1 ml/min. The methanol concentration ranged from 0 to 80%. The compounds were dissolved in NaOH 0.1 N, water or acetone and applied to the column in 5 μl volumes. All solutions were first filtered to reduce contamination. The experiments were performed at room temperature (20-22°C). The retention times were expressed as log capacity factor (k'), where $k' = (t_x - t_o)/t_o$.

R_M, $R_{M\,C18}$ and log P values. The R_M, $R_{M\,C18}$ and log P values listed in Table 1 and 2 were described previously (4).

RESULTS

Log k' values as lipophilicity indexes.

The reversed-phase HPLC at pH 7.0 of the compounds of Table 1 and 2 showed that most of the compounds were not eluted, when the mobile phase was water alone. In order to obtain suitable log k' values it was necessary to add methanol to the mobile phase. Only in the case of the 14 most hydrophilic compounds, viz. no. 1,2,3,4,5,6,7,11,13,14,21, 30, 43 and 44 reliable log k' values could be obtained even at 0% methanol in the mobile phase. They were reported in Table 3 as experimental log k' values. Log k' values higher than 1.0 at 0% methanol in the mobile phase were considered to be unreliable. However, as usually shown also in TLC, for all the compounds there was a range of linear relationship between log k' values and methanol concentrations. The equations describing such linear relationship allowed the calculation of extrapolated log k' values at 0% methanol in the mobile phase for the compounds, which did not migrate with water alone.

The range of linearity between log k' values and methanol concentrations is limited by the fact, that at the lower and higher methanol concentrations all the compounds tend not to move or to migrate with the solvent front, respectively, i.e. to deviate from the linear relationship. The extrapolated log k' values were obtained from equations calculated by means of log k' values

Table 1. Lipophilicity indexes of xanthines

No.	Chemical class / Xanthines	log k' pH 7.0	R_M pH 7.0	$R_{M\,C18}$ pH 7.0	log P
1	Purine	0.70	0.25	0.50	-0.58[b]
					-0.37[c]
2	Adenine	0.88	0.35	0.83	-0.33[b]
					-0.09[c]
3	Guanine	0.49	-0.08	----	-1.28[b]
					-0.91[c]
4	Xanthine	0.40	-0.72	-0.13	-1.65[b]
		0.97[d]	0.02[a]	0.57[a]	-0.73[c]
5	1-Methylxanthine	0.70	-0.30	0.42	-1.25[b]
6	3-Methylxanthine	0.80	-0.08	0.40	-1.00[b]
7	7-Methylxanthine	0.77	-0.12	0.26	-1.32[b]
8	1,3-Dimethyluric acid	0.93[d]	-0.73	0.60[a]	----
			-0.08[a]		
9	Theophylline (1,3-dimethylxanthine)	1.28	0.38	1.19	-0.05[b]
					-0.02[c]
10	1,7-Dimethylxanthine (paraxanthine)	1.32	0.39	1.06	-0.92[b]
11	1,9-Dimethylxanthine	0.82	-0.21	0.28	-0.92[b]
12	Theobromine (3,7-dimethylxanthine)	1.30	0.26	0.92	-0.67[b]
					-0.78[c]
13	3,9-Dimethylxanthine	0.93	0.04	0.50	-0.67[b]
14	7,9-Dimethylxanthine	0.27	-0.73	-0.46	----

15	Caffeine (1,3,7-trimethylxanthine)	1.43	0.79	1.54	0.26[b] -0.07[c]
16	Thiocaffeine	1.75[d]	1.14	2.14	----
17	3-Isobutyl-1-methylxanthine	1.76[d]	1.03	2.36	1.41[b]
18	1,3-Diethyl-8-phenylxanthine	2.06[d]	1.45	2.95	3.10[b]
19	3-Propylxanthine (enprofylline)	1.35	0.35	1.29	0.05[b]
20	7-Propylxanthine	1.20	0.25	1.07	-0.26[b]
21	9-Propylxanthine	0.97	0.04	0.65	-0.26[b]
22	1,3-Dipropyl-8-(p-sulfophenyl)xanthine	2.15[d]	1.12 1.57[a]	2.51 3.18[a]	2.31[b]
23	1,3-Dipropyl-8-(2-amino-4-chlorophenyl)xanthine	2.50[d]	2.24	3.57	4.05[b]
24	7-(ß-Hydroxyethyl)theophylline (etofylline)	1.19	0.39	0.86	-1.20[b]
25	7-(ß-Chloroethyl)theophylline	1.58	1.03	1.70	0.50[b]
26	8-Phenyltheophylline	1.73[d]	1.11	2.08	2.05[b]
27	8-(p-Sulfophenyl)theophylline	1.37[d]	0.15 0.52[a]	1.26 1.50[a]	0.19[b]
28	8-Cyclopentyltheophylline	1.82	1.03	2.55	2.16[b]
29	8-Cyclopentyl-1,3-dipropylxanthine	2.39[d]	1.94	3.61	4.28[b]

a: measured at pH 1.2
b: CLOGP
c: experimental octanol/water log P
d: log k' calculated from eqns. 2 and 3

Table 2. Lipophilicity indexes of adenosines

No.	Chemical class Adenosines/Guanosines	log k' pH 7.0	R_M pH 7.0	R_{MC18} pH 7.0	log P
30	Adenosine	0.99	0.24	0.42	-1.23[c]
31	2-Chloroadenosine	1.14	0.19	0.66	-0.34[e]
32	2-Phenylaminoadenosine	1.63	0.96	1.83	1.87[e]
33	6-Methyladenosine	1.21	0.41	0.80	-0.36[e]
34	6-Cyclopentyladenosine	1.83	1.12	2.29	1.11[e]
35	6-Cyclohexyladenosine	1.76	1.14	2.44	1.67[e]
36	6-Phenyladenosine	1.64	0.96	1.86	1.62[e]
37	6-Phenylethyladenosine	1.99	1.30	2.94	1.74[e]
38	6-(2-Phenylisopropyl)-adenosine	1.99	1.34	2.87	2.05[e]
39	6-Benzyladenosine	1.91	0.93	1.98	1.34[e]
40	5'-N-Methylcarboxamidoadenosine	1.37	0.41	0.72	-1.20[e]
41	5'-N-Ethylcarboxamidoadenosine	1.13	0.67	1.13	-0.67[e]
42	5'-N-Cyclopropylcarboxamidoadenosine	1.60	0.89	2.02	-0.84[e]
43	Guanosine	0.96	-0.38	-0.16	-1.85[c]
44	1-Methylisoguanosine	0.96	-0.33	0.08	----

c: experimental log P in octanol/water
e: log P calculated (see ref.4)

determined with various methanol concentrations, according to the lipophilicity of the test compounds. The ranges of methanol concentrations and the HPLC equations were listed in Table 3, where a and b are the intercept and slope with their standard errors, respectively; r is the correlation coefficient. The experimental log k' values of the 14 most hydrophilic compounds and the intercepts a=log k' of the remaining compounds were also reported in Table 1 and 2 in order to be correlated with other lipophilicity indexes. The 14 most hydrophilic compounds showed a linear relationship between log k' values and methanol concentrations ranging from 0 to 20%-50%. For more lipophilic compounds, ranges of increasing methanol concentrations were used. In Table 3 the compounds were listed in order of increasing lipophilicity. The validity of the extrapolation technique is shown by eq. 1 which describes a very good correlation between the experimental log k' values at 0% methanol of the 14 most hydrophilic compounds and the a= log k' values, calculated for the same compounds over a wider range of methanol concentrations (Table 3).

$$\log k'_{extrap} = 0.053(\pm 0.027)+0.953(\pm 0.034) \log k'_{exp} \quad (1)$$

(n=14; r=0.992; s=0.028; F=765.6; P<0.005)

The slopes of Table 3 are mostly constant with a mean value of -0.029±0.001

Table 3. HPLC equations of xanthines and adenosines

Cpd.	Methanol% range	HPLC equation a=log k'	b	r	log k' exp.
14	0-25	0.288 (±0.031)	-0.015 (±0.002)	0.983	0.27
4	0-20	0.477 (±0.151)	-0.040 (±0.011)	0.928	0.40
3	0-20	0.495 (±0.018)	-0.024 (±0.001)	0.997	0.49
1	0-20	0.715 (±0.051)	-0.034 (±0.004)	0.988	0.70
5	0-20	0.729 (±0.083)	-0.044 (±0.006)	0.981	0.70
7	0-20	0.794 (±0.055)	-0.041 (±0.004)	0.990	0.77
11	0.20	0.843 (±0.025)	-0.024 (±0.002)	0.989	0.82
6	0-20	0.850 (±0.106)	-0.044 (±0.008)	0.969	0.80
2	0-20	0.895 (±0.042)	-0.032 (±0.003)	0.991	0.88
13	0-20	0.893 (±0.046)	-0.040 (±0.004)	0.987	0.93
43	0-20	0.946 (±0.038)	-0.048 (±0.003)	0.997	0.96
44	0-50	0.965 (±0.163)	-0.024 (±0.005)	0.966	0.96
21	0-30	0.966 (±0.032)	-0.033 (±0.002)	0.996	0.96
30	0-20	1.042 (±0.082)	-0.029 (±0.006)	0.959	0.99
41	15-60	1.128 (±0.081)	-0.018 (±0.002)	0.975	----
31	15-60	1.140 (±0.156)	-0.022 (±0.004)	0.941	----
24	15-50	1.189 (±0.147)	-0.025 (±0.004)	0.973	----
20	15-50	1.197 (±0.195)	-0.026 (±0.006)	0.929	----
33	15-50	1.214 (±0.053)	-0.026 (±0.002)	0.994	----
9	15-40	1.276 (±0.166)	-0.032 (±0.006)	0.951	----
12	10-30	1.302 (±0.077)	-0.043 (±0.004)	0.989	----
10	20-50	1.316 (±0.161)	-0.028 (±0.004)	0.976	----
19	15-50	1.346 (±0.072)	-0.026 (±0.002)	0.992	----
40	15-50	1.367 (±0.115)	-0.029 (±0.003)	0.979	----
15	15-50	1.433 (±0.181)	-0.031 (±0.005)	0.958	----
25	30-60	1.585 (±0.128)	-0.025 (±0.003)	0.981	----
42	40-70	1.602 (±0.078)	-0.022 (±0.001)	0.996	----
32	30-70	1.634 (±0.110)	-0.025 (±0.002)	0.980	----
36	40-80	1.640 (±0.202)	-0.022 (±0.003)	0.968	----
35	40-80	1.756 (±0.485)	-0.023 (±0.008)	0.861	----
28	30-60	1.821 (±0.174)	-0.025 (±0.004)	0.969	----
34	30-80	1.829 (±0.094)	-0.025 (±0.002)	0.992	----
39	30-80	1.906 (±0.188)	-0.026 (±0.003)	0.970	----
37	40-80	1.993 (±0.244)	-0.026 (±0.004)	0.967	----
38	40-80	1.991 (±0.128)	-0.025 (±0.002)	0.986	----

Relationships between lipophilicity indexes.

In the first step of our work the log k' values were correlated with the R_M and R_{MC18} values previously obtained (4). The linear relationships are described by eqns. 2-3, which were calculated with all the available experimental data of Table 1 and 2.

$$\log k' = 0.921(\pm 0.037) + 0.763(\pm 0.056) R_M \quad (2)$$

(n=34; r=0.922; s=0.173; F=182.0; P<0.005)

$$\log k' = 0.736(\pm 0.043) + 0.458(\pm 0.030) R_{MC18} \quad (3)$$

(n=33; r=0.940; s=0.148; F=234.4; P<0.005)

As previously discussed, (4) at pH 7.0 all the compounds of Table 1 and 2 should be in their unionized form, except compounds no. 4,8,22 and 27, for which also their R_M and R_{MC18} values at pH 1.2 were determined. Therefore in calculating eqns. 2 and 3, the R_M, R_{MC18} and log k' values at pH 7.0 for compound no. 4 were not used. Since the log k' values of compounds 8,16,17,18,22,23,26,27 and 29 had not been measured, they were obtained from eqns. 2 and 3. In a similar way a log k' value for the unionized species was calculated for compound 4. It is to be noted that also for compounds 8, 22 and 27 the R_M and R_{MC18} values at pH 1.2 were used. The log k' values reported in Table 1 are the mean of the two log k' values calculated from eqns. 2 and 3 for each compound.

Finally the relationship between log k' and log P values was

described. The CLOGP values of all the xanthine derivatives were used in calculating eq. 4.

$$\log k' = 1.299(\pm 0.035) + 0.281(\pm 0.022) \log P \quad (4)$$

(n=40; r=0.900; s=0.217; F=162.9; P<0.005)

In eq. 4 compound 42 shows the highest deviation from linearity. It has been already pointed out the tendence to deviation from the linear relationship between R_M or R_{MC18} and log P values of compounds 40,41 and 42 characterized by a carboxamido group in the sugar moiety (4). In fact the correlation coefficient of eq. 5, calculated with the exclusion of compound 42, resulted to be somewhat better than that of eq. 4.

$$\log k' = 1.283(\pm 0.033) + 0.288(\pm 0.020) \log P \quad (5)$$

(n=39; r=0.917; s=0.201; F=196.8; P<0.005)

In our previous paper in correlating R_M and log P values we had used the experimental log P values of compounds 1,2,3,4,9,12 and 15, instead of their CLOGP values. The CLOGP values of the above 7 compounds allowed the calculation of eq. 6, which is very similar to eq. 5.

$$\log k' = 1.272\ (\pm 0.035) + 0.291\ (\pm 0.022) \log P \quad (6)$$

n=39; r=0.905; s=0.215; F=166.7; P<0.005)

DISCUSSION AND CONCLUSIONS

The present work shows that good correlations exist between the HPLC data and the TLC or HPTLC chromatographic indexes. In fact

eqns. 2 and 3 account for 85 and 88% of the total variance in the data (r^2). As a consequence one can say that the ΔG changes underlying the chromatographic processes involved in the determination of R_M, R_{MC18} and log k' values should be linearly related. Furthermore eqns. 2 and 3 showed their practical usefulness in that they made it possible to calculate the log k' values of some compounds for which these data were missing. The calculated log k' values as well as those derived from the chromatographic measurements were then used in the final step of this work, i.e. in describing the correlation between HPLC data and the experimental or calculated octanol/water partition coefficients. The correlation coefficient of eq. 5 is 0.917, explaining 84% of the total variance in the data. In our previous paper of this series (4) the best equations describing the relationships R_M/log P and R_{MC18}/log P explained 87 and 91% of the total variance, respectively. One can conclude that the reversed-phase TLC and HPLC systems are very similar in describing the lipophilic character of the compounds. As a final remark we want to draw the attention on the problem of the dependence of log k' values on the organic modifier concentration in the mobile phase. As a matter of fact, the slopes of the HPLC equations reported in Table 3 are fairly constant, suggesting a series of parallel

straight lines. However some deviations from parallelism are observed in the xanthines series, particularly among the most hydrophilic compounds. Such deviations could be due to structural features affecting the sensitivity of the retention process to the variations of methanol concentrations in the mobile phase. In our opinion this problem should deserve a careful study, as it could bear a general meaning.

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