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DETERMINATION OF PARTITION COEFFICIENTS OF VERY HYDROPHOBIC COMPOUNDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON GLYCERYL-COATED CONTROLLED-PORE GLASS

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SUMMARY

Reversed-phase high-performance liquid chromatography of more than 80 compounds with glyceryl-coated controlled-pore glass as the stationary phase and a mixture of methanol and water as the mobile phase showed that the capacity factor (k') correlates well with the partition coefficient between octanol and water (P_{oct}). This method is very efficient for the determination of P_{oct} for compounds with $\log P_{\text{oct}} > 5$. A general method for the determination of P_{oct} by high-performance liquid chromatography in this way is proposed.

INTRODUCTION

The hydrophobicity of biologically active compounds is known to affect their biological responses. The partition coefficient (P) of a compound between a water-immiscible organic solvent and water is a good measure of its hydrophobicity¹. In investigations on quantitative structure-activity relationships (QSAR), the general rules that govern the hydrophobicity of organic compounds have been studied extensively, and the value of $\log P$ between octanol and water has sometimes been determined with the use of the hydrophobic substituent coefficient², π , or the hydrophobic fragmental constant³, f , for simple compounds. However, the value determined in this way is not always correct when there is a strong electronic or steric effect caused by the introduction of a substituent group⁴. Hence it is safer to determine the $\log P$ value directly by an experimental procedure. The shaking-flask method, generally adopted as a standard method for the determination of $\log P$, is time consuming and tedious⁵, and is unsuitable for the accurate determination of $\log P$ values greater than 4 (ref. 6). Therefore, a simple and accurate procedure is required for the measurement of $\log P$ values.

Recently, reversed-phase high-performance liquid chromatography (RP-

HPLC) has been applied to the determination of $\log P$ (ref. 7). The capacity factor (k') in RP-HPLC is correlated with the value of $\log P$ between octanol and water determined by the shaking-flask method ($\log P_{\text{oct}}$) as shown in the equation⁸

$$\log P_{\text{oct}} = a \log k' + b \quad (1)$$

where a and b are constants characteristic of a certain partition system. The k' value is determined by RP-HPLC by the relationship

$$k' = (t_R - t_0)/t_0 \quad (2)$$

where t_R and t_0 are the retention times at a certain flow-rate of the biologically active compound and an unretained substance, respectively.

RP-HPLC on an octanol-coated column is very useful, as the properties of the stationary phase are very similar to those of octanol. For this purpose, octanol is adsorbed as the stationary phase on silica gel⁹ or on octadecylsilylated silica gel (ODS)^{10,11} and octanol-saturated buffer solution is used as the mobile phase. In this instance, the slope (a in eqn. 1) for the linear relationship between $\log P_{\text{oct}}$ and $\log k'$ was found to be very close to unity, indicating that $\log k'$ is directly related to $\log P_{\text{oct}}$ ⁹⁻¹¹. However, there are some experimental limitations to this procedure for determining the exact retention time (t_R) of a compound with a high $\log P_{\text{oct}}$, viz., very low solubility in water or the mobile phase, and a long retention time associated with a broad chromatographic peak. Note that increase in one log unit of P_{oct} results in about a 10-fold increase in t_R when a in eqn. 1 is unity. To overcome these difficulties, it is desirable to perform RP-HPLC with a mixture of water and organic solvent such as methanol and acetonitrile as the mobile phase, and with a column giving a slope a in eqn. 1 of less than unity, but still retaining the properties of octanol for a wide variety of compounds. There are two advantages of using a mixture of water and organic solvent: it affords sufficient solubility of highly hydrophobic compounds and a smaller t_R value than that obtained in the absence of an organic solvent.

There have been some reports on the chromatography of chemically bonded hydrocarbons on silica gel, such as ODS. Good linearity was observed between $\log P_{\text{oct}}$ and $\log k'$ with penicillins¹², cephalosporins¹² and propranolols¹³. However, the method has not been used for compounds with $\log P_{\text{oct}}$ values of more than about 3.

This paper reports the usefulness of glyceryl-coated controlled-pore glass (gly-CPG) as a stationary phase in RP-HPLC for the determination of the $\log P_{\text{oct}}$ values of compounds with a wide range of such values. A general method for the determination of P_{oct} by RP-HPLC is also described.

EXPERIMENTAL

N-Phenylsuccinimides were kindly supplied by Dr. Chiyoza Takayama, Sumitomo Chemical Co. (Osaka, Japan). 3'-Substituted N-phenylanthranilates were donated by Drs. Shuichi Ikawa and Eiichi Fujihira, Taisho Pharmaceutical Co. (Tokyo, Japan). All other chemicals were commercial products and were used without further purification.

RP-HPLC was carried out with a Tri-Roter-II solvent delivery system (JASCO, Tokyo, Japan) connected with a Uvidec 100-II ultraviolet detector (JASCO)

operated mostly at 210 nm. The column (50 cm \times 2.1 mm I.D.) was packed with gly-CPG (Electro-Nucleonics, Fairfield, NJ, U.S.A., Type gly00075, 200–400 mesh). Chromatography of only the neutral forms of acidic compounds was achieved by using an aqueous solution of phosphoric acid (0.03 *M*) of pH 2.2 containing various amounts of methanol as the mobile phase. For determination of partition coefficients test compounds were dissolved in methanol at concentrations of about 0.2 mg/ml and 0.5–4.0 μ l of the solution was injected on to the column, together with potassium iodide for determination of t_0 , and eluted at a flow-rate of 0.3–2.0 ml/min.

RESULTS AND DISCUSSION

Effect of methanol during chromatography

RP-HPLC of various compounds was performed using gly-CPG as the stationary phase with a mixture of water (phosphoric acid, pH 2.2) and methanol as the mobile phase. Fig. 1 shows the effect of the methanol concentration in the mobile phase on the capacity factor, k' , defined by eqn. 2. In all instances $\log k'$ decreased linearly with increase in the concentration of methanol at least up to 30% of methanol. A similar linear relationship has been observed on chromatography on an ODS column eluted with water containing methanol or acetonitrile^{14,15}. The results showed that with increase in the concentration of the organic modifier in the aqueous mobile phase, $\log k'$ decreased gradually, finally reaching a constant level. Thus, the retention time (t_R) on chromatography with gly-CPG would also become constant when the concentration of methanol is increased much more.

The linear relationship between $\log k'$ and the methanol concentration (C) in Fig. 1 is expressed by the equation

$$\log k' = \log k'_0 + m C \quad (3)$$

where m is the slope of the straight line in Fig. 1 and k'_0 corresponds to the capacity factor in the absence of methanol from the mobile phase. As shown in Fig. 1, k'_0 for

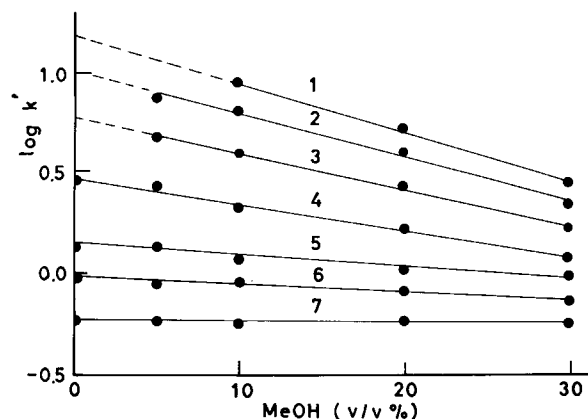


Fig. 1. Effect of methanol (MeOH) concentration on $\log k'$. 1, Flufenamic acid; 2, N-phenylantranilic acid; 3, diphenyl ether; 4, diphenyl ketone; 5, chlorobenzene; 6, methyl benzoate; 7, phenol.

hydrophobic compounds, such as flufenamic acid, N-phenylanthranilic acid and diphenyl ether, could not be determined directly owing to the low solubility of these compounds in acidic aqueous solutions. For these compounds, we determined k'_0 by extrapolating the straight line to zero methanol concentration.

The capacity factor (k') is defined by the equation¹⁶

$$k' = \frac{(X)_s}{(X)_m} \cdot \frac{V_s}{V_m} \quad (4)$$

where $(X)_s$ and $(X)_m$ are the concentrations of the solute X in the stationary (s) and mobile (m) phase, respectively, and V_s/V_m is the volume ratio of solvent to the stationary phase. According to the solubility parameter theory¹⁷, the distribution of the solute X between two phases is related to the solubility parameters of the component X (δ_x), stationary phase (δ_s) and mobile phase (δ_m) and also the molar volume of X (\bar{V}_x), as shown in the equation

$$\log \left[\frac{(X)_s}{(X)_m} \right] = \bar{V}_x \cdot \frac{(\delta_x - \delta_m)^2 - (\delta_s - \delta_x)^2}{2.3 RT} \quad (5)$$

In this study, the mobile phase consisted of two components, water (1) and methanol (2). In this instance, the solubility parameter of the mobile phase (δ_m) is expressed by the equation

$$\delta_m = (1 - C') \delta_1 + \delta_2 C' \quad (6)$$

where C' is the volume fraction of methanol in the mobile phase. Under conditions where C' is small, the following relationship applies¹⁸:

$$\log k' = \log \left(\frac{V_s}{V_m} \right) + \frac{\bar{V}_x}{2.3 RT} [(\delta_x - \delta_1)^2 - (\delta_s - \delta_x)^2] - \frac{2\bar{V}_x}{2.3 RT} (\delta_x - \delta_1) (\delta_2 - \delta_1) C' \quad (7)$$

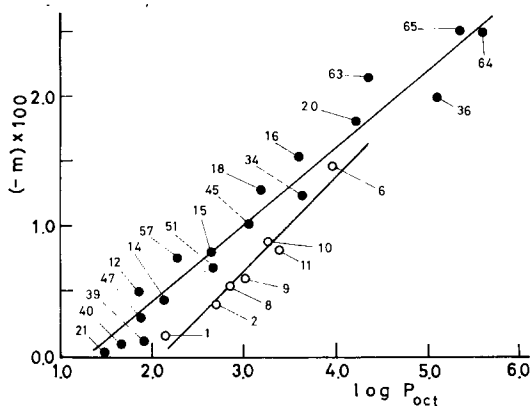


Fig. 2. Relationship between $-m$ in eqn. 3 and $\log P_{0ct}$. Numbers correspond to those for the compounds listed in Table I. O, Non-H-bonders; ●, H-bonders.

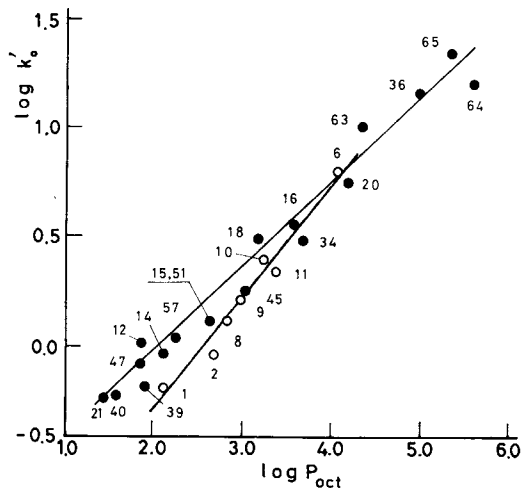


Fig. 3. Relationship between $\log k'_0$ and $\log P_{\text{oct}}$. Numbers correspond to those for the compounds listed in Table I. \circ , Non-H-bonders; \bullet , H-bonders.

The first two terms in eqn. 7 are constant in the absence of methanol, and their sum corresponds to the capacity factor (k'_0). The last term in eqn. 7 depends on the concentration of methanol (C') with a coefficient of $2\bar{V}_x(\delta_x - \delta_1)(\delta_2 - \delta_1)/2.3RT$. Thus, eqn. 7 is equivalent to eqn. 3, which explains why $\log k'$ decreased linearly with increase in the concentration of methanol up to 30% in the mobile phase, as shown in Fig. 1.

Relationship between chromatographic properties and partition coefficient

From the relationship shown in eqn. 3, k'_0 and the slope m were determined for various compounds, and these values were plotted as a function of the partition coefficient in the octanol-water system (P_{oct}). Fig. 2 shows the relationship between $-m$ and $\log P_{\text{oct}}$, and Fig. 3 shows a plot of $\log k'_0$ versus $\log P_{\text{oct}}$. The compounds used are listed in Table I. It is clear from these figures that both parameters change linearly with $\log P_{\text{oct}}$. These linear relationships are expressed by the equations

$$m = -5.81 (\pm 1.07) \times 10^{-3} \log P_{\text{oct}} + 7.83 (\pm 3.63) \times 10^{-3} \quad (8)$$

$(n = 24, r = -0.922, s = 0.003)$

and

$$\log k'_0 = 3.90 (\pm 0.04) \times 10^{-1} \log P_{\text{oct}} - 8.79 (\pm 1.21) \times 10^{-1} \quad (9)$$

$(n = 24, r = 0.979, s = 0.094)$

where n is the number of compounds, r is the correlation coefficient and s is the standard deviation. The figures in parentheses are the 95% confidence intervals. These relationships are improved when plotted separately for two groups of compounds: compounds capable of forming a hydrogen bond ("H-bonders", e.g., phenols

and benzoic acids) and those incapable of forming a hydrogen bond ("non-H-bonders", e.g., alkyl- and halobenzenes). The relationships are as follows:

For H-bonders:

$$m = -5.57 (\pm 0.99) \times 10^{-3} \log P_{\text{oct}} + 5.90 (\pm 3.44) \times 10^{-3} \quad (10)$$

$$(n = 17, r = -0.952, s = 0.002)$$

$$\log k'_0 = 3.75 (\pm 0.29) \times 10^{-1} \log P_{\text{oct}} - 8.07 (\pm 1.00) \times 10^{-1} \quad (11)$$

$$(n = 17, r = 0.991, s = 0.070)$$

For non-H-bonders:

$$m = -7.12 (\pm 1.81) \times 10^{-3} \log P_{\text{oct}} + 1.45 (\pm 0.56) \times 10^{-2} \quad (12)$$

$$(n = 7, r = -0.976, s = 0.001)$$

$$\log k'_0 = 5.48 (\pm 1.44) \times 10^{-1} \log P_{\text{oct}} - 1.42 (\pm 0.45) \quad (13)$$

$$(n = 7, r = 0.975, s = 0.079)$$

These results indicate that gly-CPG recognizes a difference in the compounds in terms of their ability to form a hydrogen bond. In Figs. 2 and 3, it should be noted that good linear relationships still hold for $\log P_{\text{oct}} > 5$. The determination of such high partition coefficients is extremely difficult by the conventional shaking-flask method. From Figs. 2 and 3, $\log k'_0$ and m are expressed as functions of $\log P_{\text{oct}}$ by the equations

$$\log k'_0 = a' \log P_{\text{oct}} + b' \quad (14)$$

and

$$m = a'' \log P_{\text{oct}} + b'' \quad (15)$$

From eqns. 3, 14 and 15, we obtain the following equation:

$$\log k'' = (a' + a''C) \log P_{\text{oct}} + (b' + b''C) \quad (16)$$

Eqn. 16 corresponds to eqn. 1 at a certain methanol concentration, C . As shown in Fig. 1, $\log k'$ decreases linearly with increase in methanol concentration according to eqn. 3. Hence the relationship between $\log k'$ and the concentration of methanol, and that between $\log k'$ and partition coefficient P_{oct} , can be depicted schematically as shown in Fig. 4. Fig. 4 shows that $\log P_{\text{oct}}$ can be determined by RP-HPLC using any of the following calibration graphs: (i) m versus $\log P_{\text{oct}}$, based on eqn. 15, (ii) $\log k'_0$ versus $\log P_{\text{oct}}$, based on eqn. 14, and (iii) $\log k'$ determined at a certain methanol concentration ($\log k'_p$) versus $\log P_{\text{oct}}$, based on eqn. 1. Of these three methods, the last is the most practically useful, as the solubility of highly hydrophobic compounds is a limiting factor in chromatography. In this instance, the methanol concentration must be as low as possible, because with a lower methanol concentration the $\log k'$ value is larger, as can be seen from eqn. 16.

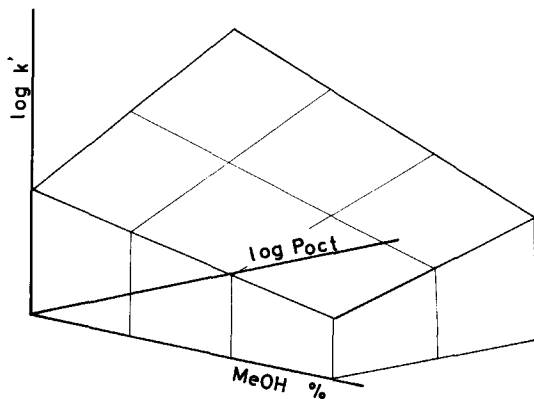


Fig. 4. Dependence of $\log k'$ on methanol concentration and $\log P_{\text{oct}}$.

Determination of partition coefficient by RP-HPLC

The above results indicate that the partition coefficient ($\log P_{\text{oct}}$) can be determined from k' at a certain methanol concentration of less than 30% in the mobile phase by RP-HPLC with gly-CPG as the stationary phase. We measured the k' values at 10% methanol ($k'_{10\%}$) for about 80 compounds. These compounds consisted of phenols, benzoic acids, N-phenylanthranilates, N-phenylsuccinimides, other miscellaneous H-accepting compounds (such as cyano- and nitrobenzene) and non-H-bonding compounds. The hydrophobicities ($\log P_{\text{oct}}$) of these compounds ranged between 1.08 and 5.62. Table I shows the $\log k'_{10\%}$ and $\log P_{\text{oct}}$ values of these compounds. The correlations between $\log k'_{10\%}$ and $\log P_{\text{oct}}$ for these compounds are listed in Table II according to their chemical structures.

In all instances, $\log k'_{10\%}$ is correlated linearly and highly significantly with $\log P_{\text{oct}}$. These relationships are almost the same with all chemical classes except non-H-bonders, which give a regression line with a lower slope. These relationships are shown in Fig. 5.

The correlation for all of the compounds, expressed by eqn. 23, is very high, but it is improved by omission of non-H-bonders (*cf.*, eqn. 24). Note that all the correlations from eqns. 17–24 are significant at more than the 99% confidence level. These results indicate that $\log P_{\text{oct}}$ can be determined exactly by RP-HPLC. When the partition coefficient is determined by RP-HPLC (the value is referred to as P_{HPLC}), it is more accurate to obtain calibration graphs with compounds belonging to the same class as the sample compounds. In Table I, $\log P_{\text{HPLC}}$ values calculated by eqns. 17–22 are listed according to chemical structures. These values are almost the same as those determined by the conventional method for all compounds, including very hydrophobic compounds, such as flufenamic acid ($\log P_{\text{oct}} = 5.62$), 3'-chloro-N-phenylanthranilic acid ($\log P_{\text{oct}} = 5.57$) and mefenamic acid ($\log P_{\text{oct}} = 5.37$).

Next, we determined P_{HPLC} for various compounds for which the P_{oct} values were not determined from the $k'_{10\%}$ values on the gly-CPG column. Table III lists the $\log P_{\text{HPLC}}$ values for these compounds determined from the corresponding correlations in Table II. The P_{HPLC} values are compared with the partition coefficients, $\log P_{\text{cal}}$, determined from the hydrophobic substituent coefficients (π). As can be seen, the $\log P_{\text{HPLC}}$ values are very similar to the $\log P_{\text{cal}}$ values in all instances.

TABLE I

CAPACITY FACTORS IN RP-HPLC ($k'_{10\%}$) AND PARTITION COEFFICIENTS (P_{oct} AND P_{HPLC}) OF VARIOUS COMPOUNDS

No.	Compound	Log $k'_{10\%}$	Log P_{oct}^*	Log P_{HPLC}^{**}	Δ^{***}
<i>(I) Non-H-bonders:</i>					
1	Benzene	-0.42	2.13	2.18	-0.05
2	Toluene	-0.13	2.69	2.62	0.06
3	Ethylbenzene	0.22	3.15	3.16	-0.01
4	Isopropylbenzene	0.38	3.66	3.40	0.25
5	Naphthalene	0.42	3.59	3.46	0.12
6	Diphenyl	0.74	3.95	3.95	0.00
7	Fluorobenzene	-0.40	2.27	2.21	0.06
8	Chlorobenzene	0.08	2.84	2.94	-0.10
9	Bromobenzene	0.12	2.99	3.00	-0.01
10	Iodobenzene	0.43	3.25	3.48	-0.23
11	1,4-Dichlorobenzene	0.40	3.38	3.43	-0.05
<i>(II) H-Acceptors:</i>					
12	$\text{C}_6\text{H}_5\text{-NO}_2$	-0.28	1.85	1.99	-0.14
13	-CN	-0.42	1.56	1.66	-0.10
14	$-\text{CO}_2\text{CH}_3$	-0.20	2.12	2.17	-0.05
15	$-\text{CO}_2\text{C}_2\text{H}_5$	-0.05	2.64	2.53	0.11
16	$-\text{CO}_2\text{C}_6\text{H}_5$	0.38	3.59	3.54	0.04
17	$-\text{COCH}_3$	-0.46	1.73	1.56	0.16
18	$-\text{COC}_6\text{H}_5$	0.28	3.18	3.30	-0.12
19	$-\text{OCH}_3$	-0.26	2.11	2.03	0.07
20	$-\text{OC}_6\text{H}_5$	0.65	4.21	4.17	0.03
<i>(III) Phenols:</i>					
21	H-	-0.45	1.48	1.54	-0.06
22	2- CH_3 -	-0.23	1.95	1.99	-0.04
23	4- CH_3 -	-0.24	1.96	1.97	-0.01
24	3- CF_3 -	0.08	2.95	2.61	0.34
25	3- C_2H_5 -	-0.11	2.40	2.23	0.17
26	4- C_2H_5 -	-0.13	2.26	2.19	0.07
27	4- C_6H_5 -	0.32	3.20	3.10	0.10
28	3-F-	-0.21	2.15	2.03	0.12
29	4-F-	-0.23	2.07	1.99	0.08
30	3-Cl-	0.05	2.68	2.55	0.13
31	4-Cl-	0.03	2.65	2.51	0.14
32	4-Br-	0.18	2.86	2.81	0.05
33	2,4- Cl_2 -	0.34	3.08	3.14	-0.06
34	2,4,6- Cl_3 -	0.51	3.62	3.48	0.14
35	2,3,4,6- Cl_4 -	0.90	4.10	4.27	-0.17
36	2,3,4,5,6- Cl_5 -	1.30	5.12	5.08	0.04
37	4- CH_3CO -	-0.43	1.35	1.58	-0.23
38	4- $\text{C}_6\text{H}_5\text{CO}$ -	0.23	3.07	2.91	0.16
39	4- NO_2 -	-0.19	1.91	2.07	-0.16
40	4-CN-	-0.32	1.66	1.80	-0.14
41	4- CH_3O -	-0.41	1.57	1.62	-0.05
42	3- CH_3CO_2 -	-0.59	1.23	1.26	-0.03
43	4- $\text{CH}_3\text{O}_2\text{C}$ -	-0.28	1.96	1.88	0.08
44	4- $\text{C}_2\text{H}_5\text{O}_2\text{C}$ -	-0.13	2.35	2.19	0.16
45	4- $\text{C}_3\text{H}_7\text{O}_2\text{C}$ -	0.11	3.04	2.66	0.38
46	2-CHO-	-0.38	1.65	1.68	-0.03

TABLE I (continued)

No.	Compound	Log $k'_{10\%}$	Log P_{oct}^*	Log P_{HPLC}^{**}	Δ^{***}
<i>(IV) Benzoic acids:</i>					
47	H-	-0.26	1.87	1.95	-0.08
48	3-F-	-0.21	2.15	2.05	0.10
49	4-F-	-0.24	2.07	1.99	0.08
50	3-Cl-	0.05	2.68	2.57	0.11
51	4-Cl-	0.03	2.65	2.53	0.12
52	3-Br-	0.18	2.87	2.83	0.04
53	4-Br-	0.18	2.86	2.83	0.03
54	3-I-	0.38	3.13	3.23	-0.10
55	4-I-	0.35	3.14	3.17	-0.03
56	3-CH ₃ -	-0.02	2.37	2.43	-0.06
57	4-CH ₃ -	-0.02	2.27	2.43	-0.16
58	3-NO ₂ -	-0.30	1.83	1.87	-0.04
59	4-NO ₂ -	-0.33	1.89	1.81	0.08
60	4-CN-	-0.46	1.56	1.55	0.01
61	2-HO-	-0.10	2.25	2.27	-0.02
62	2-CH ₃ CO ₂ -	-0.55	1.23	1.36	-0.13
<i>(V) N-Phenylanthranilates:</i>					
63	H-	0.78	4.36 [§]	4.55	-0.19
64	3'-CF ₃ -	1.21	5.62 [§]	5.47	0.15
65	2',3'-(CH ₃) ₂ -	1.23	5.37 [§]	5.52	-0.15
66	3'-CH ₃ -	0.87	4.88 [§]	4.74	0.14
67	3'-Cl-	1.27	5.57 [§]	5.60	-0.03
68	3'-NO ₂ -	0.72	4.57 [§]	4.42	0.15
69	3'-HO-	0.30	3.49 [§]	3.51	-0.02
70	3'-CH ₃ O-	0.78	4.56 [§]	4.55	0.01
71	3'-CH ₃ CO-	0.70	4.31 [§]	4.37	-0.06
<i>(VI) N-Phenylsuccinimides:</i>					
72	3-CF ₃ -	-0.52	1.26 ^{§§}	1.35	-0.09
73	4-CF ₃ -	-0.54	1.45 ^{§§}	1.30	0.15
74	3-n-C ₃ H ₇ -	-0.45	1.54 ^{§§}	1.52	0.02
75	3,5-(CF ₃) ₂ -	-0.15	2.46 ^{§§}	2.25	0.21
76	3,5-(CH ₃) ₂ -	-0.60	1.08 ^{§§}	1.16	-0.08
77	4-Br-	-0.58	1.18 ^{§§}	1.20	-0.02
78	3-I-	-0.48	1.36 ^{§§}	1.45	-0.09
79	3,5-Cl ₂ -	-0.30	1.90 ^{§§}	1.88	0.02
80	2,3,5-Cl ₃ -	-0.07	2.40 ^{§§}	2.44	-0.04
81	3,5-Br ₂ -	-0.22	2.12 ^{§§}	2.12	0.00
82	3,4,5-Cl ₃ -	0.13	2.80 ^{§§}	2.92	-0.12

* Partition coefficient between octanol and water. Values taken from ref. 19.

** Partition coefficient between octanol and water calculated from log $k'_{10\%}$ with eqns. 17-22 according to chemical class.

*** $\Delta = \log P_{oct} - \log P_{HPLC}$.

§ Taken from ref. 20.

§§ Taken from ref. 21.

The above results show that the RP-HPLC method is useful for the determination of P_{oct} , and can be used for very hydrophobic compounds. Tanaka and Thornton²² reported that k' values between 0.2 and 25 can be determined accurately by RP-HPLC. If this range is adopted as the efficient range of k' values, log P_{oct} values of

TABLE II
 LINEAR REGRESSION DATA FOR PLOT OF $\log k'_{10\%}$ VERSUS $\log P_{\text{oct}}$

$\log P_{\text{oct}} = a \log k'_{10\%} + b$. Figures in parentheses are the 95% confidence intervals of the corresponding constants.

No.*	Chemical class	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	<i>s</i>	Eqn. No.
I	Non-H-bonders	1.53 (0.26)	2.83 (0.10)	11	0.984	0.110	17
II	H-acceptors	2.35 (0.25)	2.65 (0.09)	9	0.993	0.117	18
III	Phenols	2.08 (0.13)	2.50 (0.06)	26	0.988	0.144	19
IV	Benzoic acids	2.01 (0.19)	2.47 (0.05)	16	0.987	0.092	20
V	N-Phenylanthranilates	2.16 (0.36)	2.87 (0.33)	9	0.983	0.134	21
VI	N-Phenylsuccinimides	2.42 (0.33)	2.61 (0.13)	11	0.984	0.110	22
VII	I-VI	2.26 (0.09)	2.58 (0.04)	82	0.984	0.191	23
VIII	II-VI	2.31 (0.08)	2.56 (0.04)	71	0.990	0.162	24

* See Table I.

up to about 6.5 can be determined from $\log k'_{10\%}$ values by gly-CPG column chromatography. For the determination of the P_{HPLC} values of more hydrophobic compounds, the chromatographic conditions must be adjusted so as to reduce k' to within the above range. There are three methods of doing this: (i) to use a higher

TABLE III
 COMPARISON OF CHROMATOGRAPHICALLY DETERMINED $\log P_{\text{HPLC}}$ VALUES AND CALCULATED $\log P_{\text{cal}}$ VALUES

Compound	$\log k'_{10\%}$ *	$\log P_{\text{HPLC}}$ **	$\log P_{\text{cal}}$ ***	Δ §
<i>Phenols:</i>				
4-C ₂ H ₅ -	-0.16	2.12	2.48	0.36
4-C ₆ H ₅ -	0.65	3.76	3.46	-0.30
<i>Benzoic acids:</i>				
4-C ₂ H ₅ -	0.13	2.73	2.77	0.04
4-i-C ₃ H ₇ -	0.27	2.89	3.07	0.18
4-C ₂ H ₅ O-	-0.02	2.43	2.25	-0.18
4-C ₆ H ₅ -	0.85	4.18	4.23	0.05
<i>H-acceptors:</i>				
C ₆ H ₅ CO ₂ C ₃ H ₇	0.10	2.88	3.14	0.26 [^]
C ₆ H ₅ CO ₂ C ₄ H ₉	0.33	3.42	3.64	0.22

* Capacity factor determined with 10% methanol solution as mobile phase.

** Partition coefficient in RP-HPLC determined from eqns. 18-20 according to chemical class.

*** Partition coefficient determined from the hydrophobic substituent coefficient (π).

§ $\Delta = \log P_{\text{cal}} - \log P_{\text{HPLC}}$.

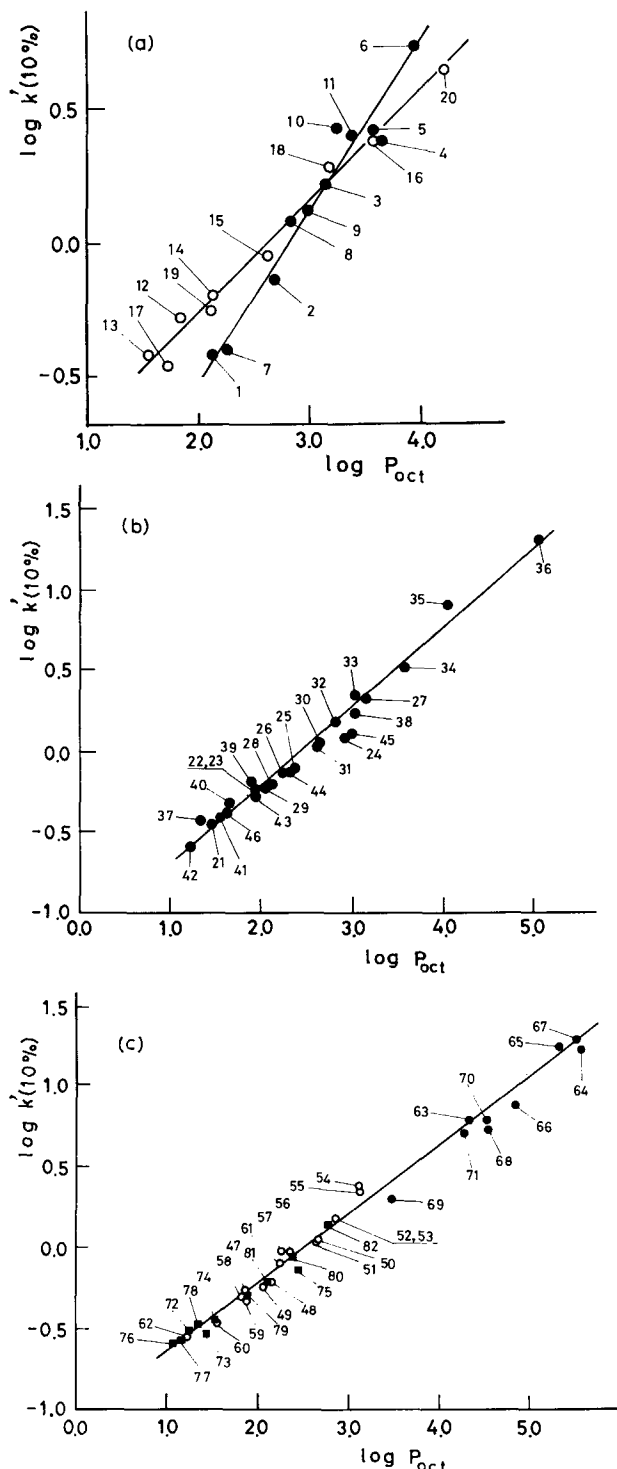


Fig. 5. (a) Relationship between $\log k'_{10\%}$ and $\log P_{oct}$. ●, Non-H-bonders; ○, H-acceptors. (b) Relationship between $\log k'_{10\%}$ of phenols and $\log P_{oct}$. (c) Relationship between $\log k'_{10\%}$ and $\log P_{oct}$. ○, Benzoic acids; ■, N-phenylsuccinimides; ●, N-phenylanthranilates. Numbers correspond to those for the compounds listed in Table I.

flow-rate of the mobile phase, (ii) to use a shorter column and (iii) to use a higher methanol concentration in the mobile phase. Changing the flow-rate or column length to optimize the chromatographic conditions is sometimes difficult, because the t_0 value become too small to determine exactly; in practice, the lower limit of t_0 is about 1 min. In the third method, it is preferable to perform the chromatography with as low a concentration of methanol as possible, as higher k' value gives a more accurate estimate of P_{oct} , as shown in Fig. 4.

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