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# REVERSED-PHASE LIQUID CHROMATOGRAPHY

# II. DETERMINATION OF PARTITION COEFFICIENTS OF BARBITURATES AND UREA HERBICIDES*

B. RITTICH*
*Research Institute of Animal Nutrition, 691 23 Pohořelice, near Brno (Czechoslovakia)*
and
H. DUBSKÝ
*Department of Forensic Medicine, Faculty of Medicine, J. E. Purkyně University, Tvrdého 2a, 662 99 Brno (Czechoslovakia)*
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## SUMMARY

Experimental $R_M$ values for a series of barbiturates were determined using reversed-phase high-performance liquid chromatography. Statistically significant linear relationships were found between these $R_M$ values and Hansch's $\pi$ parameters. Relationships were also obtained between $R_M$ values and partition coefficients determined in the system diethyl ether–dimethylformamide–water (2:1:1) and partition coefficients determined using gas–liquid chromatography. Hansch's $\pi$ parameters for anilines were correlated with $R_M$ values of some substituted urea derivatives.

## INTRODUCTION

A frequently used approach to the assessment of quantitative relationships between biological activity and chemical structure is Hansch's analysis [1,2]. An important part of this analysis is the determination of the partition coefficient in a *n*-octanol–water reference system and as defined[1] by

$$\pi = \log P_x - \log P_H \quad (1)$$

where $\pi$ is Hansch's parameter, $P_x$ is the partition coefficient of a substituted compound and $P_H$ is the partition coefficient of the corresponding unsubstituted compound.

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In previous work[3] we have studied the relationship between the $R_M$ values of phenols, as measured by different chromatographic techniques, and Hansch's parameter $\pi$. We confirmed the validity of Collander's relationship[4]

$$\log P = bR_M + a \tag{2}$$

which holds for closely related development systems where the polar phase is water. It has been suggested[5–9] that high-performance liquid chromatography (HPLC) with bonded hydrocarbon stationary phases may be suitable for determining partition coefficients, and other workers[10,11] have studied whether it is convenient to determine partition coefficients by means of gas chromatography (GC).

The aim of our study was to investigate the relationship between the partition coefficients of barbiturates and urea herbicides, determined in different separation systems, and the corresponding $R_M$ values, as measured by reversed-phase HPLC. We have also tested the suitability of GC for the determination of the partition coefficients of barbiturates.

## EXPERIMENTAL

### *Chemicals and equipment*

Samples of barbiturates (gifts from n. p. Léčiva, Prague, Czechoslovakia) were of analytical-reagent grade. The urea herbicides were monuron, diuron (DuPont, Wilmington, DE, U.S.A.), monolinuron, linuron (Hoechst, Frankfurt/M, G.F.R.), chlorbromuron, methobromuron (Ciba, Basle, Switzerland) and methoxuron (Sandoz, Basle, Switzerland). Solvents were redistilled before use.

Chromatography was performed on a Varian LC 8500 liquid chromatograph equipped with a UV flow detector ($\lambda_{max.}$ = 254 nm). The MicroPak CH-10 column (25 cm × 2.2 mm) (E. Merck, Darmstadt, G.F.R.) was packed with silica gel having an octadecyl chemically bonded non-polar phase (particle size 10 $\mu$m). Also used were: a Pye Unicam Type GCV gas chromatograph with flame ionization detector (FID) and glass columns (200 cm × 2 mm) packed with either 3% OV-1 on Diatomite CQ (0.100–0.120 mm) or 3% OV-17 on Diatomite CQ (0.100–0.120 mm); a Packard Model 419 gas chromatograph with FID and glass column (200 cm × 3 mm) packed with 3% NPGS + 0.75% TA on Chromaton N AW DMCS (0.100–0.125 mm). Before packing, all the columns were silanized.

In liquid chromatography the samples were injected by use of a 50-$\mu$l Hamilton 705 syringe, while in gas chromatography a 1-$\mu$l SGE Type 5 BL-RD-3 syringe was employed.

### *Conditions*

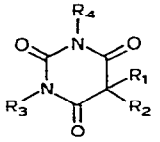
Separations on the MicroPak CH-10 column were carried out with methanol–water and dioxan–water mixtures of varying compositions as the mobile phases. The flow-rate was 60 ml/h.

In gas chromatography the temperatures of the columns, injector and of the detector were 200, 240 and 230°C, respectively. The flow-rate of the carrier gas (nitrogen) was 40 ml/min for the 3% OV-1 packing, 55 ml/min for 3% OV-17 and 60 ml/min for 3% NPGS + 0.75% TA.

In liquid chromatography the samples were dissolved in the mobile phase to give concentrations of barbiturates of *ca.* 1 mg/ml and of urea herbicides of *ca.* 0.1 mg/ml, and 5–10 μl were immediately injected by syringe. In gas chromatography the barbiturate samples were taken from an ethanolic solution (0.1 mg/ml) and 0.5 μl were injected by syringe.

## RESULTS AND DISCUSSION

The capacity factors, $k'$, of barbiturates (1) and urea herbicides (2) were calculated according to[12]

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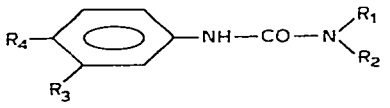


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$$R_M = \log k' = \log (V_R - V_0)/V_0 \quad (3)$$

where $V_R$ is the elution volume of a compound and $V_0$ the elution volume of an unretained compound.

We have examined the relationship between the $R_M$ (I) and $R_M$ (II) values of barbiturates measured in the mobile phases methanol–water (1:4) and dioxan–water (1:4) respectively, and the $R_M$ (III) values reported by Tjaden *et al.*[13] (the values were measured on a column of a short-chain chemically bound stationary phase, *cf.*, eqns. 4 and 5). Hemetsberger *et al.*[14] showed that there are no significant differences in chromatographic behaviour between bonded short-chain *n*-alkane packings and octadecylsilyl (ODS) types of packings.

We then studied the relationship between the values $R_M$ (I) and $R_M$ (II) and $R_M$ (IV) values calculated from the data of Baker *et al.*[6]; the latter were measured on a μBondapak $C_{18}$ column (300 × 3.9 mm) using methanol–water (2:3) as the mobile phase (pH 7). The regression coefficients calculated (eqns. 6 and 7) are statistically highly significant, which shows that in the above case the $R_M$ values measured on various commercial phases are readily comparable. Other workers[15–17] have also compared retention data measured on various types of reversed phases. On the basis of the retention data for polycyclic aromatic hydrocarbons, Ogan and Katz[15] concluded that a chromatographic analysis accomplished on one $C_{18}$ column cannot be always duplicated on a $C_{18}$ column from a different manufacturer. For further evaluation of this problem it would be necessary to perform comparisons of the chromatographic behaviour of different groups of compounds on various commercial reversed phases.

Next we investigated the relationship between $R_M$ (I), $R_M$ (II) and the logarithms of the partition coefficients, log $P$ (I) determined in *n*-octanol–water[18,19] (eqns. 8 and 9) and log $P$ (II) determined in diethyl ether–dimethylformamide–water (2:1:1)[20] (eqns. 10 and 11). Values of the partition coefficients are given in Table I.

The relationships obtained may be summarized as follows

| *Equation* | *n* | *r* | *s* | *No.* |
|---|---|---|---|---|
| $R_{M}$ (I) = 0.783 $R_M$ (III) + 0.007 | 9 | 0.978 | 0.106 | (4) |
| $R_{M}$ (II) = 0.637 $R_M$ (III) + 0.058 | 9 | 0.970 | 0.068 | (5) |
| $R_{M}$ (I) = 0.760 $R_M$ (IV) + 0.451 | 9 | 0.953 | 0.106 | (6) |
| $R_{M}$ (II) = 0.615 $R_M$ (IV) + 0.415 | 9 | 0.955 | 0.076 | (7) |
| $R_{M}$ (I) = 0.450 log $P$ (I) + 0.165 | 10 | 0.967 | 0.082 | (8) |
| $R_{M}$ (II) = 0.435 log $P$ (I) + 0.089 | 9 | 0.973 | 0.055 | (9) |
| $R_{M}$ (I) = 1.117 log $P$ (II) + 0.564 | 8 | 0.895 | 0.160 | (10) |
| $R_{M}$ (II) = 1.095 log $P$ (II) + 0.480 | 7 | 0.803 | 0.180 | (11) |

where $n$ = number of compounds in the set, $r$ = regression coefficient and $s$ = standard deviation. All the relationships are statistically significant ($P < 0.01$). The regression coefficients calculated for eqns. 10 and 11, using the partition coefficients determined in diethyl ether–dimethylformamide–water (2:1:1), are, however, lower than those for eqns. 8 and 9 where the partition coefficients were determined in *n*-octanol–water. When evaluating these regression equations it must be remembered that the investigated compounds were partly dissociated in the mobile phase.

Reversed paper[21] or thin-layer chromatography[22–25] has also been used for measuring partition coefficients. In previous work[3] we have shown that there is a statistically significant linear relationship between the $R_M$ values as measured by HPLC and PC or TLC.

We have also examined the relationship between the partition coefficients of

TABLE I

PARTITION COEFFICIENTS OF BARBITURATES DETERMINED BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

Systems: I = MicroPak CH-10, methanol–water (1:4), flow-rate 60 ml/h; II = MicroPak CH-10, dioxan–water (1:4), flow-rate 60 ml/h; III = methyl silica, methanol–water (1:3), taken from Tjaden *et al.*[13].

| *Compound* | *Substituents* | | | $R_M$ *values* | | | *log P (I)** | *log P (II)*** |
|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | *I* | *II* | *III* | | |
| Amobarbital | $C_2H_5$ | $CH_2CH(CH_3)_2$ | H | 1.195 | 1.052 | 1.544 | 2.07 | – |
| Butobarbital | $C_2H_5$ | $(CH_2)_3CH_3$ | H | 0.917 | 0.802 | 1.170 | 1.65 | – |
| Pentobarbital | $C_2H_5$ | $CH(CH_3)(CH_2)_2CH_3$ | H | 1.195 | 1.035 | 1.526 | 2.03*** | 0.532 |
| Allobarbital | $CHCH=CH_2$ | $CHCH=CH_2$ | H | 0.595 | 0.544 | 0.866 | 1.05*** | 0.230 |
| Phenobarbital | $C_2H_5$ | $C_6H_5$ | H | 0.748 | 0.693 | 0.783 | 1.42 | −0.071 |
| Barbital | $C_2H_5$ | $C_2H_5$ | H | 0.398 | 0.368 | 0.554 | 0.65 | −0.097 |
| Eudan | $CH_3$ | $C_6H_5$ | H | 0.602 | 0.536 | 0.763 | – | – |
| Hexobarbital | $CH_3$ |  | $CH_3$ | 1.073 | 0.869 | 1.310 | 1.92 | 0.556 |
| Cyclobarbital | $C_2H_5$ |  | H | 0.938 | 0.809 | 1.190 | 1.86*** | 0.146 |
| Aprobarbital | $C_3H_5$ | $C_3H_7$ | H | 0.748 | 0.637 | – | 1.15 | 0.161 |
| Thiopentobarbital§ | $C_2H_5$ | $CH(CH_3)(CH_2)_2CH_3$ | H | 1.404 | – | – | 3.00 | 0.851 |

* The partition coefficient in the system *n*-octanol–water, taken from Fujita[18].

** The partition coefficient in the system diethyl ether–dimethylformamide–water (2:1:1), taken from Melichar *et al.*[20].

*** From Hansch *et al.*[19].

§ In formula I the atom of oxygen is substituted by sulphur.

barbiturates as determined by reversed-phase liquid chromatography, $R_M$ (I), $R_M$ (II), and by gas chromatography on 3% NPGS + 0.75% TA, $R_M$ (A), on 3% OV-17, $R_M$ (B), and on 3% OV-1, $R_M$ (C) (Table II). The partition coefficients of barbiturates determined by gas chromatography were calculated also according to eqn. 3.

Eqns. 12–17 give the relationships obtained for fixed phases of varying polarity:

| *Equation* | *n* | *r* | *s* | *No.* |
|---|---|---|---|---|
| $R_M$ (I) = 0.111 $R_M$(A) + 0.756 | 9 | 0.147 | 0.287 | (12) |
| $R_M$ (II) = 0.113 $R_M$(A) + 0.644 | 9 | 0.186 | 0.232 | (13) |
| $R_M$ (I) = 0.289 $R_M$(B) + 0.537 | 9 | 0.370 | 0.270 | (14) |
| $R_M$ (II) = 0.230 $R_M$(B) + 0.495 | 9 | 0.363 | 0.220 | (15) |
| $R_M$ (I) = 0.376 $R_M$(C) + 0.646 | 9 | 0.486 | 0.253 | (16) |
| $R_M$ (II) = 0.310 $R_M$(C) + 0.575 | 9 | 0.494 | 0.205 | (17) |

None of the relationships is statistically significant. The value of the regression coefficient increases as the polarity of the fixed phase decreases and it is highest for the non-polar phase OV-1 (eqns. 16 and 17).

In GC, with sufficiently dilute solutions, Henry's law is applicable. The volatility of compounds is then determined mainly by intermolecular forces between the molecules of separated compounds and those of the stationary phase. In contrast, in liquid chromatography there are interactions involving both the stationary and mobile phases. For this reason, GC is considered unsuitable for the determination of partition coefficients that bear a linear relation to the partition coefficients determined in *n*-octanol–water. This conclusion is in agreement with the results of Stoubaut *et al.*[10] who made a similar comparison in O-alkyl O-aryl phenylphosphonothioates. On the other hand, Boček[11] reported that true partition coefficients can be measured in the systems water–nitrogen and oleyl alcohol–nitrogen using the method described by Conder and co-workers[26,27].

TABLE II
PARTITION COEFFICIENTS OF BARBITURATES DETERMINED BY GAS CHROMATOGRAPHY
Conditions as in the text.
Columns: A = 3% NPGS + 0.75% TA on Chromaton N AW DMCS; B = 3% OV-17 on Diatomite CQ; C = 3% OV-1 on Diatomite CQ.

| *Compound* | $R_M$ *value* | | |
|---|---|---|---|
| | *A* | *B* | *C* |
| Allobarbital | 0.895 | 0.885 | 0.487 |
| Amobarbital | 0.879 | 1.028 | 0.565 |
| Aprobarbital | 0.788 | 0.903 | 0.301 |
| Barbital | 0.586 | 0.669 | 0.000 |
| Butobarbital | 0.827 | 0.985 | 0.367 |
| Cyclobarbital | 1.507 | 1.616 | 1.054 |
| Hexobarbital | 0.845 | 1.404 | 0.845 |
| Pentobarbital | 0.981 | 1.091 | 0.636 |
| Phenobarbital | 1.710 | 1.693 | 1.054 |

Separation of differently substituted derivatives of urea using reversed-phase liquid chromatography was also studied (see Table III). The $R_M$ values of urea herbicides measured in the mobile phase dioxan–water (1:4) were correlated with Hansch's parameters for anilines[28,29] (Fig. 1). When calculating Hansch's parameter $\pi$ for anilines with two ring substituents, we took account of the additivity of substitution increments. This simplification was based on the work of Colin *et al.*[17] who for so-called pseudohomologous series of polymethylbenzenes and polymethylphenols obtained quasi-linear relationships between the number of carbon atoms and log $k$.

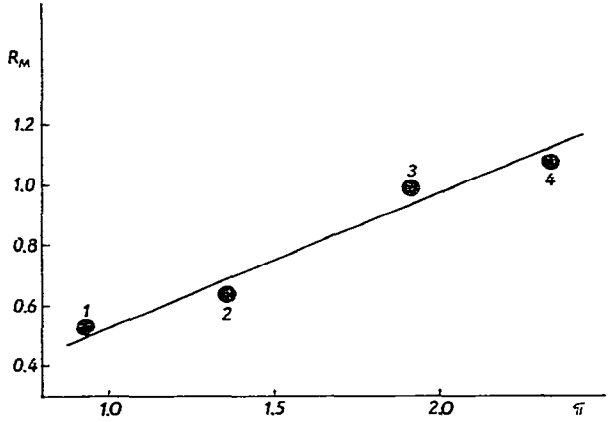


Fig. 1. Relationship between the $R_M$ values of urea herbicides and Hansch's parameter $\pi$ for anilines: 1 = monolinuron; 2 = metabromuron; 3 = linuron; 4 = chlorbromuron. MicroPak CH-10, dioxan–water (1:4), flow-rate 60 ml/h.

TABLE III
PARTITION COEFFICIENTS OF UREA HERBICIDES DETERMINED BY REVERSED-PHASE LIQUID CHROMATOGRAPHY
Systems: I = MicroPak CH-10, methanol–water (3:7), flow-rate 60 ml/h; II = MicroPak CH-10, dioxan–water (1:4), flow-rate 60 ml/h.

| *Compound* | *Substituents* | | | | *$R_M$ value* | | $\pi$* |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | *I* | *II* | |
| Monuron | $CH_3$ | $CH_3$ | H | Cl | 0.501 | 0.367 | 0.93 |
| Diuron | $CH_3$ | $CH_3$ | Cl | Cl | 0.983 | 0.794 | 1.91 |
| Monolinuron | $CH_3$ | $OCH_3$ | H | Cl | 0.666 | 0.535 | 0.93 |
| Chlorbromuron | $CH_3$ | $OCH_3$ | Cl | Br | 0.833 | 1.059 | 2.34 |
| Methobromuron | $CH_3$ | $OCH_3$ | H | Br | 0.760 | 0.628 | 1.36 |
| Methoxuron | $CH_3$ | $CH_3$ | Cl | $OCH_3$ | 0.412 | 0.235 | 1.03 |
| Linuron | $CH_3$ | $OCH_3$ | Cl | Cl | 1.135 | 0.982 | 1.91 |

* Hansch's parameter for anilines, taken from Leo *et al.*[28] and Fujita *et al.*[29].

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