Liquid Chromatographic Determination of Lipophilicity with Application to a Homologous Series of Barbiturates

STEPHEN TOON *, JOACHIM MAYER, and MALCOLM ROWLAND ˣ

Received June 17, 1982, from the *Department of Pharmacy, University of Manchester, Manchester M13 9PL, England.* Accepted for publication May 9, 1983. *Present address: School of Pharmacy, University of Washington, Seattle, WA 98105.

Abstract □ A graphical method for determining the lipophilicity of the members of a homologous series of barbituric acids, from a consideration of their reverse-phase HPLC retention data, is described. The HPLC parameter used as the index of lipophilicity, R_Q, is shown not only to form excellent correlations with the more commonly employed indices of lipophilicity, R_m and log P, but also to have a predictive capability for those log P values that had not previously been determined experimentally.

Keyphrases □ Lipophilicity—homologous series of barbiturates, determination by the HPLC parameter R_Q □ Barbiturates—lipophilicity, determination by the HPLC parameter R_Q □ HPLC—use of the parameter R_Q to determine lipophilicity, homologous series of barbiturates

Since Meyer (1) and Overton (2) first postulated a relationship between the biological response elicited by a drug substance and its lipophilic character, medicinal chemists have sought rapid and reliable methods of determining molecular lipophilicity. The octanol-water partition coefficient (P), classically being determined using the "shake-flask" method (3), has been the index of lipophilicity most commonly employed in correlations between molecular structure and biological response (4, 5). The "shake-flask" approach to the determination of lipophilic character has been shown, however, to have many inherent practical difficulties associated with it (6–8), and as a consequence alternative methods of determining P or other indices of lipophilicity have been sought.

Many of the alternatives have been derived from TLC (9–12) and high-performance liquid chromatography (HPLC) (13–16), but in nearly all instances problems arise when characterizing highly lipophilic or hydrophilic molecules. The potential of the HPLC-based term R_Q (17) as an index of molecular lipophilicity was explored using a homologous series of barbituric acids. Special reference was paid to the correlation of R_Q with the more commonly accepted indices of lipophilicity, log P and R_m, the latter being experimentally determined from reverse-phase TLC studies within the series.

EXPERIMENTAL

The barbituric acids comprising the series (Table I) were obtained either from commercial sources or synthetically by condensing the respective diethyl 2-alkyl-2-ethylmalonate with urea in the presence of sodium ethoxide. The synthetic barbituric acids were characterized by ^{1}H-NMR, elemental analyses, and melting point determinations. All were calculated to be >99% pure.

Liquid Chromatographic Analysis and the Determination of R_Q Values—The analytical system has been previously outlined (17). The basis of the HPLC assay is that the un-ionized barbiturates are separated using a reverse-phase column[1], and then by the postcolumn infusion of pH 10 buffer, the barbiturates are converted to the more strongly UV-absorbing monoanionic species, facilitating detection at 254 nm.

The acetonitrile[2] and acetic acid[3] used in the preparation of the mobile phases were HPLC and analytical grade, respectively. All water used was freshly glass-distilled. The mobile phases were deaerated prior to use by filtration under vacuum through a 0.22-μm microbial filter[4].

The barbiturates were studied individually as solutions in acetonitrile (50 μg/mL), 10 μL of which was used in the analysis, thereby facilitating the measurement of retention times of the various barbiturates (R_T) relative to the unretained solute peak (10 μL of methanol, R_0) at varying concentrations of acetonitrile in the mobile phase. To improve the retention characteristics of 5-ethylbarbituric acid, 0.05% (v/v) acetic acid was added to the mobile phase; the retention of the other members of the series was unaffected by this addition.

R_Q values were calculated at the various acetonitrile concentrations according to:

$$R_Q = \log\left(\frac{R_T - R_0}{R_T}\right) \qquad \text{(Eq. 1)}$$

where R_T is the retention time of the solute and R_0 is the retention time of the unretained solvent. Values of R_Q at 0% v/v acetonitrile (100% v/v water; R_{Q0}) and at 40% v/v acetonitrile (R_{Q40}) were derived for all of the compounds either by direct measurement or by extrapolation/interpolation of the plots of the R_Q *versus* concentration (% v/v) of acetonitrile in the mobile phase.

Thin-Layer Chromatography and Calculation of R_m Values—Silica gel[5] was spread as a water slurry onto 20 × 20-cm glass plates to a thickness of 0.25 mm. After air drying, the plates were activated by heating overnight in an oven at 110°C. The plates were predeveloped in an 1-octanol–acetone (1:9) mixture, after which the acetone was evaporated from the plates by the use of an air blower. The silica gel plates were thus coated with a layer of 1-octanol.

Solutions of the individual barbiturates in acetone (2 mg/mL) were prepared, and 30 μl of each were applied as discrete spots along the baseline, positioned 1 cm from the bottom edge of the TLC plate. The prepared plates were developed in one of seven possible solvent systems composed of differing concentrations of acetone in water (5, 15, 25, 30, 35, 37, and 40% v/v acetone in water).

The ratio of the distance traveled by a given solute (barbiturate), relative to that traveled by the solvent front, was calculated in each instance yielding R_f values, which in turn allowed the calculation of R_m values for the individual barbiturates at varying acetone concentrations according to (18, 19):

$$R_m = \log\left(\frac{1}{R_f} - 1\right) \qquad \text{(Eq. 2)}$$

Assessment of log P—A solution (5×10^{-4} M) of the compound to be investigated (compounds 1, 2, and 3; Table I) was prepared in Sörensen's buffer (pH 5.1, 0.07 M) saturated with 1-octanol. This solution was then shaken gently for 1 h with 1-octanol that had been previously washed successively with 1 M NaOH, 1 M HCl, and the Sörensen's buffer. A period of 1 h to ensure that equilibrium had been achieved is more than adequate; only a few minutes are generally needed (3). The ratio of the volumes of the two phases was chosen so that 20–60% of the solute remains in the aqueous phase after extraction. The concentration of compound in the aqueous phase, adjusted to pH 10 by addition of dilute NaOH, was determined spectrometrically (240 nm) before and after extraction. The partition coefficient (P) of the acid was calculated from the relationship:

$$P = \frac{V_a}{V_o}\left[\frac{Ca(0)}{Ca} - 1\right] \qquad \text{(Eq. 3)}$$

where $Ca(0)$ and Ca are the respective concentrations of the compound in the aqueous phase before and after extraction and V_a and V_o are the volumes of the aqueous and organic phases, respectively.

[1] Hypersil ODS 5μm; Shandon Southern Products, U.K.
[2] Acetonitrile; Rathburn Chemicals, U.K.
[3] Acetic acid; May & Baker Ltd., U.K.
[4] Millipore Corp.
[5] Kieselgel, G type 60 and Kieselgel 60 GF_{254} in a 1:1 ratio; E. Merck, West Germany.

0022-3549/84/0500-0625$01.00/0

Table I—Chromatographic and Literature Indices [a] of Lipophilicity for the Barbiturates

Key	Compound	R_m (25% v/v Acetone)	log P (Octanol–Water)	R_{Q0}	R_{Q40}
1	5-Ethylbarbituric acid	—	−1.52	−0.083	−1.215
2	5-Ethyl-5-methylbarbituric acid	−0.31	0.02	−0.002	−0.562
3	Barbital	−0.02	0.68[b]	0.051	−0.469
4	5-Ethyl-5-*n*-propylbarbituric acid	0.38	0.87[c]	0.114	−0.314
5	Butethal	0.83	1.70[c]	0.187	−0.221
6	5-Ethyl-5-*n*-hexylbarbituric acid	—	3.08[c]	0.292	−0.084
7	5-Ethyl-5-*n*-heptylbarbituric acid	—	3.64[c]	0.304	−0.040
8	5-Ethyl-5-*n*-octylbarbituric acid	—	3.85[c]	0.361	0.017
9	5-Ethyl-5-*n*-nonylbarbituric acid	—	4.13[c]	0.384	0.052
10	Pentobarbital	1.06	2.13[c]	0.190	−0.166
11	Amobarbital	1.12	2.11[c]	0.235	−0.157
12	Phenobarbital	0.48	1.42[b]	0.140	−0.244

[a] Leo *et al.* (3) reported $R_{Q40} = 0.121 \cdot \log P - 0.447$ ($n = 10, r^2 = 0.9470$); $R_{Q0} = 0.086 \cdot \log P + 0.021$ ($n = 10, r^2 = 0.9740$); $R_{Q40} = 0.280 \cdot R_m - 0.446$ ($n = 7, r^2 = 0.9470$); and $\log P = 1.465 \cdot R_m + 0.520$ ($n = 6, r^2 = 0.9619$). [b] Taken from Hansch and Leo (30). [c] Taken from Yih (31).

RESULTS AND DISCUSSION

The barbituric acid series under investigation varies widely in lipophilic character (Table I) and problems were encountered in determining TLC R_m values for the more lipophilic members of the series. Although a TLC solvent containing <40% acetone yielded a suitable migration from the baseline of the more hydrophilic homologues, hence facilitating the calculation of R_m values, elution from the baseline of the lipophilic *n*-hexyl through *n*-nonyl homologues was not afforded under such conditions. Furthermore, a solvent containing ≥40% acetone was found to strip the 1-octanol layer from the precoated plates, rendering them useless for the purpose in hand.

For those members of the series that did lend themselves to TLC analysis, a linear relationship was shown to exist between R_m and the acetone concentration (% v/v) in the developing solvent (Fig. 1). Similar relationships have previously been demonstrated for other molecular series, including the benzodiazepines and penicillins (9, 11).

Our results highlight the problem in quantitating lipophilic character of highly nonpolar compounds by TLC. Hulshoff and Perrin (20) have proposed a TLC-based method for the determination of the relative partition coefficients of very lipophilic basic compounds, based on the manipulation of developing solvent pH. While promising, this method has received only limited application (21).

Most HPLC approaches to the determination of lipophilicity are based on attempts made at formulating relationships between the capacity factor k' [$k' = (R_T - R_0)/R_0$; R_T and R_0 as previously defined] and P. The capacity factor (k') of a solute is only constant under any given set of chromatographic conditions. If k' values are to serve as the index of lipophilicity for the members of a homologous series, therefore, they should be obtained, for each homologue, under identical chromatographic conditions, an almost impossible constraint when dealing with a series of widely ranging lipophilicities.

Initial attempts at obtaining k' values for the barbituric acid series using a single isocratic HPLC system proved fruitless. The maximum possible concentration of organic solvent in the mobile phase that allowed a suitable retention time for the most hydrophilic homologue, 5-ethylbarbituric acid, was 10% v/v acetonitrile in 0.05% v/v aqueous acetic acid. Elution of the more hydrophobic homologues was not afforded under these conditions, with predicted retention times for these compounds being in excess of 10 h.

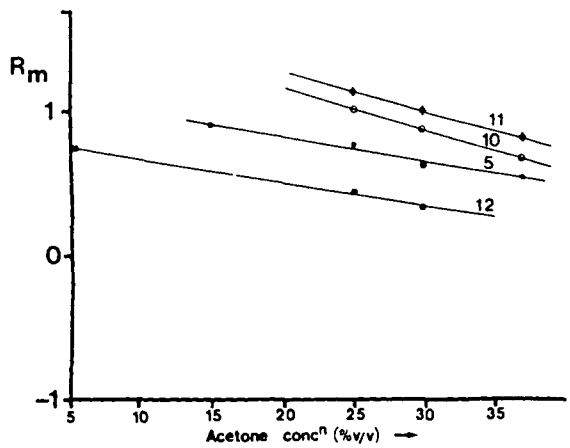

Figure 1—*Plot of* R_m *against acetone concentration in the mobile phase for various barbiturates. See Table I for key to the numbers.*

Yamana *et al.* (22) noted a linear relationship between log k' and the concentration of methanol in the HPLC mobile phase for a series of penicillins and cephalosporins. Such a relationship facilitates the calculation of k' values for the individual homologues at two or more mobile phase compositions, those which yield adequate retention times of the solute in question. These empirically determined k' values may then be extrapolated to give k' values at any desired mobile phase composition. Yamana *et al.* extrapolated their plots of log k' *versus* mobile phase composition to obtain log k' values for all members of their series at 0% v/v methanol (100% v/v water), these values in turn being shown to be highly correlated with the respective log P and R_m values.

The values of k' and log k' do not always vary linearly with mobile phase composition (17); thus, extrapolation of k' *versus* mobile phase plots is not always possible. As seen in Fig. 2, log k' for the barbiturate series is proportional to the concentration of acetonitrile in the mobile phase for many, but not all, of the homologues.

In contrast to k', we have found R_Q to vary linearly with mobile phase composition in all the cases we have examined, even when marked nonlinearity has been shown to exist between log k' and mobile phase composition (17). Plots of R_Q *versus* the concentration of acetonitrile in the mobile phase (Fig. 3) were back-extrapolated to yield R_Q values at 0% v/v acetonitrile (R_{Q0}) for all of the homologues. R_{Q0} values obtained in this manner were found to form good correlations with both TLC R_m values as well as with literature log P values (Fig. 4). The choice of a reference state of 0% v/v acetonitrile in water in relating R_Q values to other measures of lipophilicity is arbitrary. A better reference point would be one at which R_Q could be empirically determined for the maximum number of homologues, thereby reducing the number of R_Q values obtained by extrapolation, giving increased confidence in the R_Q data. A mobile phase of 40% v/v aqueous acetonitrile provides such a reference point.

Using the regression equation of R_{Q0} against log P, octanol–water partition coefficients were predicted for the two lowest homologues as being (log P) −1.52 for 5-ethylbarbituric acid and 0.02 for 5-methylbarbituric acid. As no literature values for log P were available for these two homologues, empirical determination was necessary to check the predicted values. The experimentally determined values for 5-ethylbarbituric acid and 5-ethyl-5-methylbarbituric

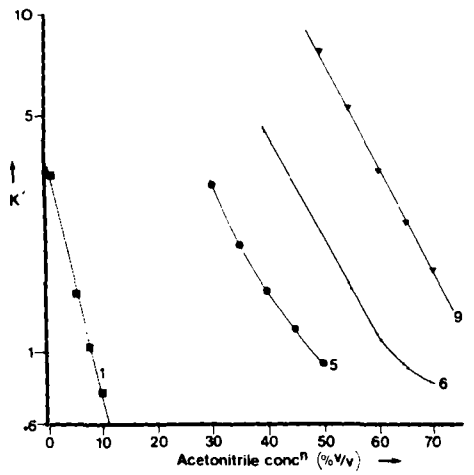

Figure 2—*Semilogarithmic plot of log* k' *against the organic modifier, acetonitrile, in the mobile phase for various barbiturates. See Table I for key to the numbers.*

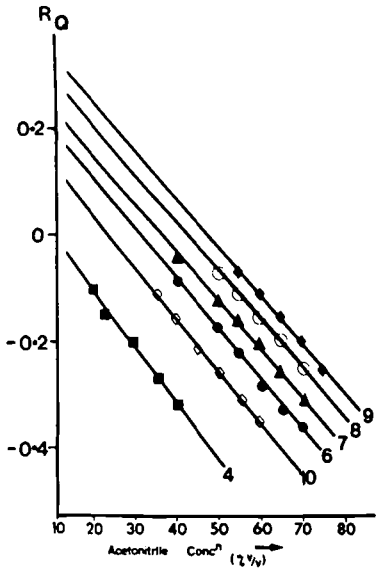

Figure 3—*Plot of* R_Q *against the organic modifier, acetonitrile, in the mobile phase for various barbiturates. See Table I for key to the numbers.*

acid were −1.26 and 0.2, respectively, illustrating the good predictive capabilities of R_Q.

Pretreatment of column packings prior to the determination of lipophilicity using an HPLC based method has been the cause of considerable debate. Several workers have capped the reverse-phase column prior to use (23), while others have coated the column with 1-octanol (24–26). Baker *et al.* (27), proposed that the ordered side chains and residual silanol groups of untreated octadecylsilane packing material best reflects a biolayer, and partition data obtained from such systems are more suitable for use in biological correlations. A commercially available untreated reverse-phase packing material was used throughout our experimental procedure. In view of the excellent correlations obtained between R_Q values and other indices of molecular lipophilicity, we feel that pretreatment of the column packing prior to use may be unnecessary.

The thermodynamic basis of HPLC-derived lipophilicity data has been the subject of several investigations (28, 29). The thermodynamic basis of R_Q, if there is one, is unclear. From a theoretical standpoint, however, we should point out that the ratio $(R_T - R_0)/R_T$ cannot exceed 1 and R_Q cannot therefore exceed 0. Several of the extrapolated R_{Q0} values (Table I) are numerically greater than zero. Even so, these R_Q values form excellent correlations with the more commonly accepted indices of lipophilicity.

HPLC offers several advantages in the determination of lipophilicity over more conventional approaches, the main ones being speed and analytical sensitivity; these advantages are inherent in the proposed parameter R_Q. Unlike k' (the HPLC term most commonly used to quantiate molecular lipophilicity), R_Q has been found to vary linearly with mobile phase composition

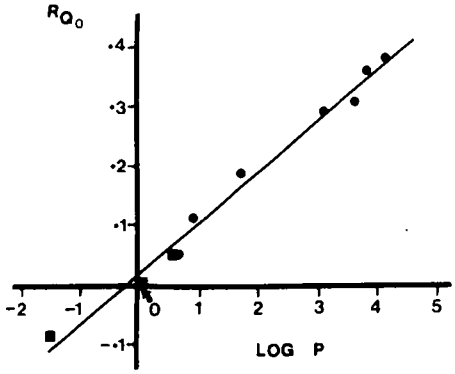

Figure 4—*Plot of* R_{Q0} *against log P for various barbiturates taken from the literature (●) or determined experimentally (■). Straight line is the line of best fit using the 10 literature log P values. See Table I for key to the numbers.*

in all cases examined. This linear correlation allows interpolation and some extrapolation from empirically determined R_Q values to values at any selected mobile phase composition. At a minimum, R_Q values need only be empirically determined at two differing mobile phase compositions to obtain any desired value of R_Q. From a practical viewpoint, we feel that as many empirically determined R_Q values as possible should be used, from solute retention data where R_T falls within the range of $1.5\text{–}15 \times R_0$. R_Q thus provides a rapidly determined index of lipophilicity which can be applied to compounds of widely ranging lipophilic character.

REFERENCES

(1) K. H. Meyer, *Arch. Exp. Pathol. Pharmacol.*, **42,** 109 (1899).

(2) E. Overton, "Studien über die Narkose," Jena, Germany, 1901.

(3) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71,** 525 (1971).

(4) C. Hansch, "Drug Design," Vol. 1, E. J. Ariens, Ed., Academic, New York, N.Y., 1971.

(5) C. Hansch, R. T. Muir, T. Fujita, P. P. Maloney, F. Geiger, and M. Streich, *J. Am. Chem. Soc.*, **85,** 2817 (1963).

(6) C. B. C. Boyce and B. V. Milborrow, *Nature (London)*, **208,** 537 (1965).

(7) T. Braumann and L. H. Grimme, *J. Chromatogr.*, **206,** 7 (1981).

(8) S. H. Unger, J. R. Crook, and J. S. Hollenberg, *J. Pharm. Sci.*, **67,** 1364 (1978).

(9) G. L. Biagi, A. M. Barbaro, M. C. Gverra, M. Babbini, M. Gaiardi, and M. Bartoletti, *J. Med. Chem.*, **23,** 193 (1980).

(10) M. Bachrata, M. Blesova, A. Schultrova, L. Grohchova, Z. Bezakova, and A. Lukas, *J. Chromatogr.*, **171,** 29 (1979).

(11) G. L. Biagi, A. M. Barbaro, M. F. Gamba, and M. C. Gverra, *J. Chromatogr.*, **41,** 371 (1969).

(12) M. Kuchar, V. Rejholec, B. Brunova, and M. Jelinkova, *J. Chromatogr.*, **195,** 329 (1980).

(13) A. Nahum and C. Horvath, *J. Chromatogr.*, **192,** 315 (1980).

(14) B. Riftich, M. Polster, and O. Kralik, *J. Chromatogr.* **197,** 43 (1980).

(15) J. F. K. Huber, C. A. M. Meyers, and J. A. R. J. Hulsman, *Anal. Chem.*, **44,** 111 (1972).

(16) S. H. Unger and C. H. Chiang, *J. Med. Chem.*, **24,** 262 (1981).

(17) S. Toon and M. Rowland, *J. Chromatogr.*, **208,** 391 (1981).

(18) E. C. Bate-Smith and R. G. Westall, *Biochim. Biophys. Acta*, **4,** 427 (1950).

(19) S. Marcinkiewicz, J. Green, and S. McHale, *J. Chromatogr.*, **10,** 42 (1963).

(20) A. Hulshoff and J. H. Perrin, *J. Chromatogr.*, **120,** 65 (1976).

(21) G. Maksay, Z. Teggery, and L. Otuos, *J. Chromatogr.*, **174,** 447 (1979).

(22) T. Yamana, A. Tsuji, E. Mizamoto, and O. Kubo, *J. Pharm. Sci.*, **66,** 747 (1977).

(23) J. M. McCall, *J. Med. Chem.*, **18,** 549 (1975).

(24) M. S. Mirlees, S. J. Moutlon, C. T. Murphy, and P. J. Taylor, *J. Med. Chem.*, **19,** 615 (1976).

(25) D. Henry, J. H. Block, J. L. Anderson, and G. R. Carlson, *J. Med. Chem.*, **19,** 619 (1976).

(26) S. H. Unger and T. F. Feverman, *J. Chromatogr.*, **176,** 426 (1979).

(27) J. K. Baker, D. O. Rauls, and R. F. Borne, *J. Med. Chem.*, **22,** 1301 (1979).

(28) E. Tomlinson, H. Poppe, and J. C. Kraak, *Int. J. Pharmaceut.*, **7,** 225 (1981).

(29) C. M. Riley, E. Tomlinson, and T. M. Jefferies, *J. Chromatogr.*, **185,** 197 (1979).

(30) C. Hansch and A. Leo, "Substituent Constants for Correlation Analysis in Chemistry and Biology," Interscience, New York, N.Y., 1979.

(31) T. D. Yih, Ph.D. Thesis, University of Nijmegen, The Netherlands (1976).

ACKNOWLEDGMENTS

We gratefully acknowledge grants from the British Science and Engineering Research Council (for S.T.) and Swiss National Science Foundation/Royal Society (for J.M.M.) and a grant-in-aid from Merck Sharp & Dohme Research Laboratories.