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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF SUBSTITUTED *p*-BENZOQUINONES AND *p*-HYDROQUINONES

II. RETENTION BEHAVIOR, QUANTITATIVE STRUCTURE–RETENTION RELATIONSHIPS AND OCTANOL–WATER PARTITION COEFFICIENTS

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SUMMARY

The retention behavior of methoxy-substituted *p*-benzoquinones and the corresponding hydroquinones in reversed-phase chromatography was examined on octylsilica and two octadecylsilica stationary phases and with five hydroorganic mobile phases containing acetonitrile, methanol or tetrahydrofuran and additionally in most cases $(NH_3OH)_3PO_4$ used as a reducing and buffering agent. The retention order of benzoquinones and hydroquinones was the same on each stationary phase with either methanol or acetonitrile as the organic modifier. On the other hand, minor differences in the retention order were observed with the various stationary phases. In all cases, satisfactory quantitative structure–retention relationships (QSRRs) were found and the data suggest that the differences in the retention behaviour of octadecylsilicas used in this study are silanophilic interactions which, together with solvophobic interaction contribute to the retention of these eluites. Further analysis showed that QSRRs of sterically crowded molecules must take into account reduced surface area available for binding. The retention data obtained with use of aqueous tetrahydrofuran as mobile phase failed to give rise to satisfactory QSRRs. This was attributed to selective solvation of eluite by tetrahydrofuran and/or nearly equipotent binding of eluite and tetrahydrofuran to stationary phase.

INTRODUCTION

Quinones and hydroquinones represent a class of compounds of great biological interest. Ubiquinones are universal mediators of electron transport. Other less widely distributed quinones are found in plants, where they serve many roles includ-

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ing that of allelochemicals for protection against predation[1–6]. Szent-Györgyi has suggested that certain methoxy-substituted *p*-quinones may have an essential but hitherto unrecognized role in maintaining metabolic electronic state in animals and the tissue concentrations of these methoxy-substituted *p*-benzoquinones and *p*-hydroquinones may play a role in maintaining the organism against degenerative disease and cancer[7–9]. The chemical and physiochemical properties of this class of molecules have been little studied particularly as far as their behavior in aqueous media is concerned[10,11]. Their chromatographic separation is encumbered by the on-column oxidation of *p*-hydroquinone[12], and probably the reverse reaction to a lesser extent, that results in unchromatographic elution profiles characterized by maxima corresponding to oxidized and reduced forms and an intervening, frequently plateauing, reaction zone.

The interest in the physiological significance of these compounds prompted us to measure their octanol–water partition coefficients and to examine the relationship between their molecular structure and retention behavior in reversed-phase chromatography and thereby gain further information on the physicochemical properties of these substances.

EXPERIMENTAL

Apparatus

Three chromatographs were used in these studies. One was assembled from two Beckman (Berkeley, CA, U.S.A.) Model 100A pumps with a system controller (Rheodyne, Berkeley, CA, U.S.A.) Model 7120 sampling valve with a 5-μl loop (Kratos, Ramsey, NJ, U.S.A.), a Model 770 UV spectrophotometer or DuPont (Wilmington, DE, U.S.A.) UV detector, a Linear Instruments (Irvine, CA, U.S.A.) Model 5555 strip-chart recorder or an Milton Roy LDC (Riviera Beach, FL, U.S.A.) integrating recorder. The second was comprised of a Perkin-Elmer (Norwalk, CT, U.S.A.) Series 10 pump, a Rheodyne Model 7010 injection valve with a 5-μl loop, a Kratos Model 773 variable-wavelength UV detector (Bioanalytical Systems, West Lafayette, IN, U.S.A.), a Model LC-4B amperometric detector and a Kipp en Zonen (Delft, The Netherlands) Model BD-41 strip-chart recorder. The third was an IBM Instruments (Danbury, CT, U.S.A.) LC/9533 ternary gradient liquid chromatograph with microcomputer-controlled operator station, a Model 9522, 254 nm UV detector and a Model 9540 chromatography data integrator. This unit was equipped with a Rheodyne Model 7125 injector and a Model 5000 Fischer Recordall recorder.

Chemicals

2-Methoxy-*p*-hydroquinone (2-MHQ), 2,6-dimethoxy-*p*-hydroquinone (2,6-DMHQ), 2,3-dimethoxy-*p*-hydroquinone (2,3-DMHQ), 2,5-dimethoxy-*p*-hydroquinone (2,5-DMHQ), 2,3,5-trimethoxy-*p*-hydroquinone (2,3,5-TMHQ) and 2,3,5,6-tetramethoxy-*p*-hydroquinone (2,3,5,6-TMHQ) and the corresponding *p*-benzoquinones were donated by G. Fodor (University of West Virginia). The acronyms of the *p*-benzoquinones are 2-MBQ, 2,6-DMBQ, 2,3-DMBQ, 2,5-DMBQ, 2,3,5-TMBQ and 2,3,5,6-TMBQ, respectively. Methanol, acetonitrile and tetrahydrofuran were chromatographic grade from Baker (Phillipsburg, NY, U.S.A.). Distilled water was obtained with a Barnstead unit in our laboratory. $(NH_3OH)_3PO_4$ was from South-

western Analytical (Austin, TX, U.S.A.). Disodium salt of ethylenediamine tetraacetic acid (Na_2EDTA) and other chemicals were reagent grade from Fisher (Fair Lawn, NJ, U.S.A.). Trimethyl chlorosilane and dimethyl octadecyl chlorosilane were obtained from Petrarch Systems (Levittown, PA, U.S.A.).

Columns

Stationary phases used included 5-μm endcapped octylsilica and octadecylsilica from IBM Instruments, 250 × 4.5 mm I.D. and 5-μm Spherisorb (PhaseSep, Hauppage, NY, U.S.A.) and lab-packed octadecylsilica prepared by reaction of 5-μm Spherisorb silica gel with dimethyl octadecyl chlorosilane and endcapping with trimethyl chlorosilane[13], the column dimensions were 250 × 4.6 mm I.D. in each case.

Operating conditions

In all cases isocratic elution was used at 1.0 ml/min flow-rate and 25°C. The column effluent was monitored at 285 or 290 nm except for the detection of 2-MBQ which was carried out at 254 nm.

The "reducing" mobile phase was prepared as follows. Hydroxylammonium phosphate and Na_2EDTA were dissolved in deionized water to yield a solution of 50 m*M* and 25 m*M* for the two components, respectively. The solution having pH 6.05 was filtered through a 0.2-μm Millipore filter and mixed with 10 or 20% (v/v) organic solvent.

The non-reducing mobile phase was a mixture of an aqueous solution containing 50 m*M* triethylamine and 16.7 m*M* EDTA and 10 or 20% (v/v) of acetonitrile. When electrochemical detection was used, the mobile phase was heated to 35°C in the reservoir and sparged with nitrogen and samples were purged by nitrogen for 5 min before injection.

Column hold-up volume was determined from the retention volume of sodium nitrate.

Determination of partition coefficient

An amount of 2 mg of the *p*-benzoquinone under investigation and 2 mg phenol, the internal standard, were dissolved in 1 ml water and allowed to equilibrate with 1 ml of 2-octanol by shaking overnight in closed 5-ml glass vials. Aliquots of 5 μl of the upper and lower phases were individually chromatographed on the IBM C_{18} column. The partition coefficient of *p*-benzoquinone, $K_{s,ow}$ was calculated from the peak areas and the known partition coefficient of the internal standard phenol $K_{p,ow}$ by the following equation

$$K_{s,ow} = K_{p,ow}R_o/R_w \quad (1)$$

where R_o and R_w are the peak area ratios of *p*-benzoquinone/phenol as measured on the chromatograms obtained with samples of the octanol (o) and water (w) phase, respectively. The oxidative instability of *p*-hydroquinones precluded the evaluation of their partition coefficients by this method.

Quantitative structure activity relationships

The parameters of eqns. 2–9 were obtained by a multiple linear regression

analysis by use of the GLM package in the SAS statistical analysis system on an IBM 370/158 computer at the Yale Computer Center.

Molecular connectivities were calculated by the method of Randić[14] as modified by Kier and Hall[15].

RESULTS AND DISCUSSION

Measurement of retention factors

It has been shown[12] that on-column oxidation of *p*-hydroquinones interferes with their chromatographic separation and encumbers the identification of peaks and the measurement of retention values. In order to lessen this effect, the data presented here were obtained with the use of the "reducing" mobile phase described in the Experimental section. Chromatograms of various *p*-hydroquinones and *p*-benzoquinones obtained on octyl- and octadecylsilica stationary phases, and the reducing mobile phase containing 10% (v/v) methanol, are shown in Figs. 1 and 2, respectively. At the concentrations employed in the eluent, $(NH_3OH)_3PO_4$ did not reduce benzoquinones palpably. The use of this mobile phase was not trouble free, however, as 2,5-DMHQ was found to be unstable and gave rise to early eluting decomposition products shortly after dissolving the sample in the reducing eluent.

The retention factors of methoxy-substituted *p*-hydroquinones and *p*-benzoquinones were measured on various silica bound hydrocarbonaceous stationary phases with different hydro-organic mobile phases and the results are given in Table I. On both octyl- and octadecylsilica stationary phases retention factors were greater with hydro-organic eluents containing 20% (v/v) methanol rather than 20% (v/v) acetonitrile in agreement with the relative eluotropic strengths of these organic modifiers[16]. Relative retentions obtained with those eluents were similar on both stationary phases. When tetrahydrofuran was used as the organic component of the mobile phase, however, a significantly different set of relative retentions was obtained. Comparison of the retention values on a given column with reducing and non-reducing

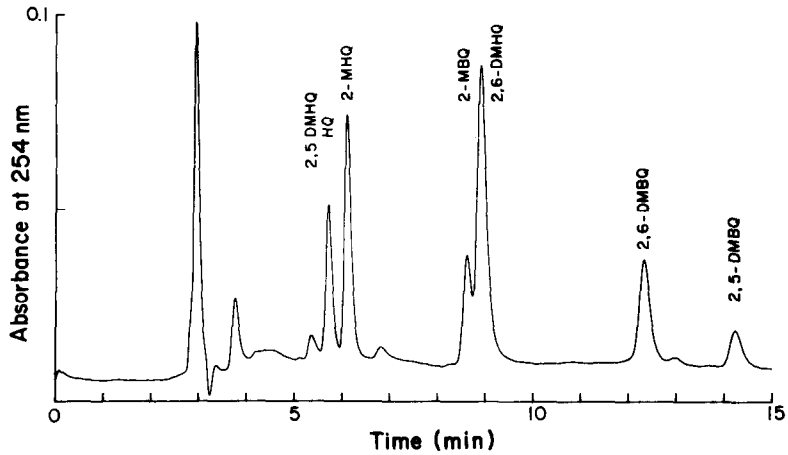

Fig. 1. Chromatogram of *p*-hydroquinone, 2-MHQ, 2,5-DMHQ, 2,6-DMHQ and the corresponding benzoquinones. Column, IBM C_8; eluent, methanol–0.050 *M* $(NH_3OH)_3PO_4$, 0.025 *M* Na_2EDTA (10:90); flow-rate 1.0 ml/min; temperature, 25°C; detection at 290 nm; sample size, 20 μl.

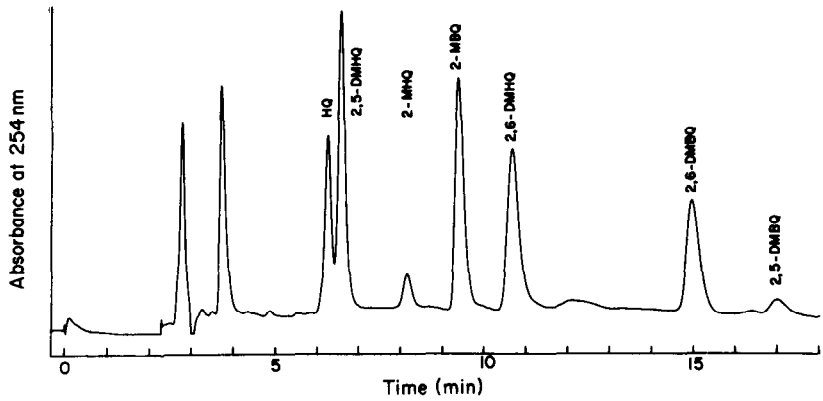

Fig. 2. Chromatogram of *p*-hydroquinone, 2-MHQ, 2,5-DMHQ, 2,6-DMHQ and the corresponding benzoquinones. Column, IBM C_{18}; eluent, methanol–0.050 *M* $(NH_3OH)_3PO_4$, 0.025 *M* Na_2EDTA (10:90); flow-rate, 1.0 ml/min; temperature, 25°C; detection at 290 nm; sample size, 20 μl.

mobile phases showed that retention factors were generally greater in the absence of $(NH_3OH)_3PO_4$ and EDTA but the retention orders were the same with both the reducing and non-reducing eluents having the same organic solvent content.

Quantitative structure–retention relationships

The retention data reported in Table I were analyzed in order to establish quantitative structure–retention relationships (QSRRs). The natural logarithm of the retention factor for each substance investigated is expressed as

$$\ln k = \ln k_0 + I_B \ln \alpha_{B-H} + I_3\tau_3 + I_5\tau_5 + I_6\tau_6 \quad (2)$$

where k_0 is the retention factor of 2-MHQ, which is taken as the parent compound, α_{B-H} is the relative retention of a *p*-benzoquinone–*p*-hydroquinone pair and τ_3, τ_5 and τ_6 represent the incremental change in the logarithmic retention factor upon substituting a methoxy group at the 3, 5 or 6 position in the molecule, respectively. The indicator variables I_3, I_5 and I_6 take the value of 0 or 1 according to the presence of methoxy groups in the respective position, and the indicator variable I_B has the value 0 or 1 for *p*-hydroquinone or *p*-benzoquinone, respectively. The parameters evaluated by multiple linear regression analysis on data obtained with various mobile and stationary phases are given in Table II.

In eqn. 2 the methoxy groups at positions 3, 5 and 6 are regarded as being different, therefore this approach could be considered as too detailed in this respect. On the other hand, the contribution of the 6-methoxy group is assumed to be the same in 6-DMBQ, 2,6-DMBQ and 2,3,5,6-TMBQ, and the neglect of *ortho* interactions may make this approach to be too crude. It was therefore of interest to examine other equations relating structure and retention factor in order to find the most satisfactory QSRR and thus expand the scope of this investigation.

By considering *ortho* interactions between methoxy groups at the 5 and 6 positions by use of the aditional term $I_{56}\tau_{56}$, eqn. 2 can be extended to obtain

$$\ln k = \ln k_0 + I_B \ln \alpha_{B-H} + I_3\tau_3 + I_5\tau_5 + I_6\tau_6 + I_{56}\tau_{56} \quad (3)$$

TABLE I

RETENTION FACTORS OF METHOXY-SUBSTITUTED *p*-HYDROQUINONES AND *p*-BENZOQUINONES

Columns: A, endcapped 5-μm octadecylsilica from IBM; B, endcapped 5-μm octylsilica from IBM; C, lab-packed endcapped octylsilica prepared from 5-μm Spherisorb. ACN = acetonitrile, MeOH = methanol, THF = tetrahydrofuran.

Eluite	*Column*								
	A					*B*	*C*		
	*20% ACN**	*20% MeOH**	*10% MeOH**	*10% MeOH***	*10% THF**	*10% MeOH***	*20% ACN**	*20% MeOH**	*10% THF**
2-MHQ	0.81	1.00	1.94	2.31	1.90	1.81	1.00	1.38	1.48
2,6-DMHQ	0.85	1.42	2.86	4.03	1.38	3.83	1.07	2.06	1.40
2,3-DMHQ	1.13	1.78	–	–	2.22	–	1.36	2.13	1.77
2,5-DMHQ	0.88	1.85	3.18	–	1.76	–	1.50	5.50	1.60
2,3,5-TMHQ	1.21	2.81	–	–	1.82	–	1.47	3.31	1.65
2,3,5,6-TMHQ	1.57	4.14	–	–	2.04	3.87	1.86	4.63	1.84
2-MBQ	1.63	1.85	2.40	4.85	0.96	6.14	2.07	3.48	1.00
2,6-DMBQ	1.70	2.61	4.14	8.63	0.88	–	2.33	5.38	1.13
2,3-DMBQ	2.83	3.81	–	–	1.76	–	3.50	6.25	1.63
2,5-DMBQ	1.45	2.59	5.14	8.16	0.72	6.93	5.54	12.4	1.03
2,3,5-TMBQ	2.78	6.67	–	–	1.57	–	3.57	4.63	1.77
2,3,5,6-TMBQ	4.64	11.0	–	–	2.82	–	5.71	12.1	3.00

* The aqueous portion contained 50 m*M* $(NH_3OH)_3PO_4$ + 25 m*M* Na_2 EDTA.
** The aqueous portion contained 50 m*M* triethylamine + 16.7 m*M* Na_2 EDTA.

where I_{56} has the value 1 if both positions are substituted and the value zero otherwise, and τ_{56} is the magnitude of τ account for this effect.

Alternatively we can assume that each methoxy group makes the same contribution to the logarithmic retention factor, τ_{OMe}, and terms $I_3\tau_3$ and $I_{56}\tau_{56}$ account for 2–3 and 5–6 *ortho* interactions, respectively. Then we obtain

$$\ln k = \ln k_0 + I_B \ln \alpha_{B-H} + I_3\tau_3 + n_{OMe}\tau_{OMe} + I_{56}\tau_{56} \quad (4)$$

where n_{OMe} is the number of methoxy groups in the molecule. When only 5–6 *ortho* interactions are significant, eqn. 4 reduces to

$$\ln k = \ln k_0 + I_B \ln \alpha_{B-H} + n_{OMe}\tau_{OMe} + I_{56}\tau_{56} \quad (5)$$

Whereas eqns. 4 and 5 imply that the two *ortho* effects are different, this may not be so. Assuming that n_{ortho} interactions between *ortho* methoxy groups give rise to the same incremental increase, τ_{ortho}, in the logarithmic retention factor we find that

$$\ln k = \ln k_0 + I_B \ln \alpha_{B-H} + n_{OMe}\tau_{OMe} + n_{ortho}\tau_{ortho} \quad (6)$$

Another approach involves the use of the topologically-based connectivity indices, ${}^0\chi^v$, ${}^1\chi^v$ and ${}^2\chi^v$, which represent the connectivities of each 1-atom, 2-atom (*i.e.* 1-bond) and 3-atom (*i.e.* 2-bond) system, respectively[14,15]. Then we can express the logarithmic retention factor as

$$\ln k = {}^0\chi^v + {}^1\chi^v + {}^2\chi^v \quad (7)$$

Since parameters α_{B-H} and τ_{OMe} are expected to entail at least as much detail about eluite molecular structure as ${}^0\chi^v$ and ${}^1\chi^v$, the latter parameters can be replaced in eqn. 7 to yield

$$\ln k = \ln k_0 + I_B \ln \alpha_{B-H} + n_{OMe}\tau_{OMe} + {}^2\chi^v \quad (8)$$

TABLE II

PARAMETERS OF QUANTITATIVE STRUCTURE–RETENTION RELATIONSHIPS ACCORDING TO EQN. 2 FOR METHOXY-SUBSTITUTED *p*-BENZOQUINONES AND *p*-HYDROQUINONES

Columns and abbreviations as in Table I.

Column	*Mobile phase*	*ln* k_0	*ln* α_{B-H}	τ_3	τ_5	τ_6	*Relative standard error*	*Correlation coefficient*
A	Water–ACN (80:20)	−0.351	0.788	0.533	0.179	0.136	16.2	0.941
	Water–MeOH (80:20)	−0.054	0.659	0.700	0.526	0.393	12.7	0.975
	Water–THF (90:10)	0.329	−0.347	0.537	0.956	−0.047	29.4	0.666
C	Water–ACN (80:20)	0.580	0.881	0.248	0.572	−0.075	20.5	0.919
	Water–MeOH (80:20)	0.619	0.844	0.166	0.900	0.075	21.6	0.905
	Water–THF (90:10)	0.678	−0.117	0.334	0.316	−0.097	22.2	0.701

TABLE III

CORRELATION COEFFICIENTS OF RETENTION DATA OF METHOXY-SUBSTITUTED *p*-BENZOQUINONES AND *p*-HYDROQUINONES BY USING VARIOUS QSRR EQUATIONS

Columns and abbreviations as in Table I.

Column	*Mobile phase*	*Correlation coefficient of data calculated by eqn.*							
		2	*3*	*4*	*5*	*6*	*7*	*8*	*9*
A	Water–ACN (80:20)	0.941	0.967	0.966	0.885	0.966	0.843	0.942	0.943
	Water–MeOH (80:20)	0.975	0.972	0.969	0.952	0.964	0.877	0.959	0.961
	Water–THF (90:10)	0.666	0.744	0.744	0.485	0.744	0.760	0.638	0.647
C	Water–ACN (80:20)	0.919	0.968	0.858	0.858	0.858	0.650	0.908	0.913
	Water–MeOH (80:20)	0.905	0.952	0.721	0.656	0.706	0.566	0.799	0.811
	Water–THF (90:10)	0.701	0.678	0.677	0.473	0.677	0.618	0.621	0.630

The above equation can be expanded by explicitly including 5–6 interactions in addition to $^2\chi^v$, so that we obtain

$$\ln k = \ln k_0 + I_B \ln \alpha_{B-H} + n_{OMe}\tau_{OMe} + I_{56}\tau_{56} + {}^2\chi^v \quad (9)$$

The data were analyzed by use of eqns. 1–9 in order to gain insight into the molecular structural information minimally required for QSRRs. For this purpose, an adequate QSRR is regarded as one in which the correlation coefficient between observed retention factors and those predicted by use of the QSRR exceeds 0.95. The concordance of the data in Table I to eqns. 2–9 were examined by use of linear regression. The correlation coefficients obtained with use of each equation on each data set are given in Table III.

Inspection of Table III reveals that none of the above equations is consistently optimal in describing the retention data. In most cases eqn. 3 gives the best results yet the results obtained by eqn. 2 are almost as good. Eqns. 4, 6 and 9 yield similar results but are generally inferior to eqns. 2 and 3. The least satisfactory fit is obtained by eqns. 5 and 8.

The results suggest that a simple model that regards the charge in retention factor with increase of methoxy group only is insufficient. For the data examined here, at least, the explicit (or implicit) consideration of *ortho* effects on hydrophobic interactions by use of the parameters τ_{ortho}, τ_{56} or τ_3 is necessary. The connectivity parameter $^2\chi^v$ may also implicitly include the effect. However, equations in which $^2\chi^v$ is used to account for the *ortho* effect are generally not as successful as those which include more molecular detail by use of the parameters τ_{ortho}, τ_3 or τ_{56}. Attempts to use $^3\chi^v$, the connectivity of all four-atom systems, did not improve the correlation between logarithmic retention factor and molecular structure as represented by connectivity index.

Analysis of the statistical significance of the parameters in the models reveals that $\ln \alpha_{B-H}$ must be included in any relationship. Thus, a minimally acceptable QSRR for these compounds must include linear combination of $\ln \alpha_{B-H}$, τ_{OMe} and τ_{ortho} for effect correction or an equivalent collection of parameters. The use of other

parameters can be envisioned. For example, we anticipate that the use of molecular surface area and ln α_{B-H} as parameters in QSRR would result in fits as good as those given by eqn. 2 or 3.

Examination of Table II shows the correlation coefficients to be high for data obtained with acetonitrile or methanol as organic cosolvent in hydroorganic mobile phase upon analysis according to eqn. 2. The results of regression analysis appropriate for these mobile phases are generally satisfactory for this rather crude model. However, by this criterion very poor results are obtained with use of 10% (v/v) tetrahydrofuran in mobile phase and two sources of this poor fit are likely. First, solvation of the various methoxy-substituted *p*-quinones by tetrahydrofuran may include steric effects in the solute–solvent interactions that are not related to eluite structure in a simple way. Second, if retention occurs bind more than one mechanism because more than one class of binding site exists. The interpretation of the retention factor increment becomes ambiguous. For example, if only two mechanisms are found then log k is given by eqn. 10:

$$\log k = \log (K_1 + K_2) \tag{10}$$

where K_1 and K_2 are the equilibrium constants (and phase ratio) for binding by mechanisms 1 and 2 respectively, *e.g.* at solvophobic sites and silanols. The logarithmic retention increment for structural feature s, τ_s, is defined by eqn. 11.

$$\tau_s = \mathrm{d}(\ln k)/\mathrm{d}(n_s) \tag{11}$$

where n_s is the frequency of structural feature s in the molecule. By combination of eqns. 10 and 11, the value of τ_s is found to be given by eqn. 12

$$\tau_s = \omega_1 \tau_{1,s} + \omega_2 \tau_{2,s} \tag{12}$$

where ω_1 and ω_2 are given as $\varphi_1 K_1/(K_1 + K_2)$ and $\varphi_1 K_2/(K_1 + K_2)$, respectively and $\tau_{1,s}$ and $\tau_{2,s}$ are the logarithmic retention increments in mechanism 1 and 2, respectively.

Thus, τ_s is given as the weighted average of the τ values appropriate to each mechanism (or site) with the weighting factor given as the fraction of eluite retained by each mechanism. The magnitude of the weighting factor is expected to change between eluites.

This could be the source of divergence between columns in the results shown in Table II. If the retention free energies on the two stationary phases are identical for each eluite, expected in the case of the same retention mechanisms, then the τ values obtained with a given mobile phase should be identical for both columns. Examination of the data in Table II shows that the τ values are distinguishable so that different retention mechanisms are likely to occur on the two columns. For the eluites under investigation, the solvophobic effect[17] is believed to govern retention, although interaction with surface silanols could also contribute to retention[18,19]. If the surface concentration of silanol binding sites available for hydrogen-bonding on the stationary phase with respect to that of the available hydrocarbonaceous sites is different between columns the weighting factors will be different between molecules

which do or do not interact with silanols. Thus the relative phase ratios, as obtained from the intercept of plots of retention factor obtained on one column *versus* that on another, will depend on the structural features of the eluites if (i) more than one type of interaction with the stationary phase occurs, and (ii) the weighting factors appropriate to each kind of interaction are not in constant proportion[20]. In view of such considerations, the results imply that the retention of hydroquinones involves more silanophilic interactions on Supelcosil C_{18} than on IBM C_{18}.

Partition coefficients

Experimentally measured and calculated partition coefficients K_{ow} and retention factors obtained with 2-MBQ, 2,3-DMBQ, 2,6-DMBQ and 2,3,5-TMBQ are presented in Table IV. Partition coefficients were calculated by

$$\log K_{ow} = \log K_{ow,HQ} + n_{OMe} \cdot \tau'_{OMe} \quad (12)$$

where $\log K_{ow,HQ}$, n_{OMe} and τ'_{OMe} are the logarithmic partition coefficients of hydroquinones, the number of methoxy groups in solute, and the logarithmic increase in partition coefficient due to methoxy group, respectively. The values of $\log K_{ow,HQ}$ and τ'_{OMe} (0.2 and -0.02, respectively) were taken from the literature[21]. Octanol–water partition coefficients were determined as described in Experimental. The partition coefficient is seen to increase as the number of methoxy groups increases and the logarithmic retention factors included in Table IV follow the same trend. However, comparison of the experimental retention factors with those calculated from the parameters obtained by linear regression of $\log k$ *vs.* $\log K_{ow}$ shows poor fit. Furthermore, linear free energy relationships developed by use of eqns. 2–9 by using $\ln K_{ow}$, instead of $\ln k'$ resulted in poor correlation coefficients ranging from 0.4 to 0.7. According to the literature, the octanol–water partition coefficient of *p*-hydroquinone exceeds that of *p*-benzoquinone[23]. Experimental data from our laboratory also suggest that the octanol–water partition coefficient is greater for the hydroquinones than for the corresponding benzoquinones. This seems to contradict, however, the general rule that reversed-phase chromatographic retention factors parallel the corresponding octanol–water partition coefficient[16,22,23]. Furthermore, the values of τ_3–τ_6 are

TABLE IV

CALCULATED AND PREDICTED OCTANOL–WATER PARTITION COEFFICIENTS AND RETENTION FACTORS ON ENDCAPPED OCTADECYLSILICA PREPARED FROM SUPELCOSIL FOR METHOXY-SUBSTITUTED *p*-BENZOQUINONES

The relationship between retention factors k and the experimental water–octanol partition coefficients is given as $\log k = 0.261 \log K_{ow} + 0.812$.

Solute	*log K_{ow} octanol–water*		*log k 20% ACN in water–octadecyl*	
	Experimental	*Calculated*	*Experimental*	*Calculated*
2-MBQ	−0.060	0.18	0.212	0.212
2,6-DMBQ	−0.056	0.16	0.230	0.216
2,3,5-TMBQ	0.220	0.14	0.440	0.440
2,3-DMBQ	0.338	0.16	0.452	0.535
2,3,5,6-TMBQ	0.413	0.12	0.667	0.596

positive in contrast to the corresponding negative π value for partitioning that has generally been observed for methoxy groups attached to benzene moiety[21]. Positive values have only been reported for methoxy groups attached to N-aromatic rings or to *meta* or *para* position in nitrobenzene. Some differences in the pattern observed for partitioning and retention can be expected in principle because partitioning is determined by the properties of solute in the bulk of the two phases, but reversed-phase retention is governed by solute interactions between a bulk mobile phase and a stationary phase surface. The discrepancy can be reconciled more satisfactorily, however, by recognizing that the partition coefficient of *p*-benzoquinone/*p*-hydroquinone exceeds one if the organic solvent is not octanol but cyclohexane or oils[21]. Therefore, the reversed-phase chromatography retention of these compounds may well be more directly analgous to partitioning between oil and water rather than between octanol and water. If so, reversed-phase chromatography retention data cannot confidently be used to estimate K_{ow} for use in quantitative structure activity relationships. However, the chromatographic results may be especially useful for prediction of behavior of biological compounds in matrices such as membranes that may provide more apolar environment than 1-octanol.

CONCLUSIONS

The retention behavior of twelve methoxy-substituted hydroquinones and benzoquinones was investigated on three alkylsilica stationary phases and three mobile phases. The octanol–water partition coefficients of the benzoquinones were also determined. The chromatographic results were examined for quantitative structure–retention relationships.

The QSRRs appropriate to use of hydro-organic eluents containing methanol or acetonitrile but not tetrahydrofuran were found to be linear functions of simple structural contributions. The peculiar behavior in eluents containing tetrahydrofuran is probably due to topospecific effects binding of tetrahydrofuran to binding sites of benzoquinones and hydroquinones.

Analysis of the retention data indicate benzoquinones are more retained than the corresponding hydroquinones and that retention increases with increase in number of methoxy groups in the molecule. The pattern of methoxy group selectivity in retention is similar to that in octanol–water partitioning. On the other hand the stronger retention of benzoquinones than that of hydroquinones is the opposite than that predicted on the basis of partitioning between octanol and water but agrees with the results obtained when cyclohexane or oil is the organic solvent. Insofar as change in reversed-phase retention with structure usually parallels the corresponding change in octanol–water partition coefficient, this result is surprising. However, the discrepancy can be reconciled if retention to be governed by interactions between the eluite and the stationary phase surface and the bulk solution and not its bulk alone. Therefore reversed-phase chromatographic data cannot be used uncritically for estimation of octanol–water partition but it may yield insight into other partitioning processes.

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