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Determination of partition coefficients of non-ionic contrast agents by reversed-phase high-performance liquid chromatography

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ABSTRACT

Reversed-phase high-performance liquid chromatography was used to measure the lipophilicities of non-ionic contrast agents. Calculated partition coefficients were correlated with the capacity factors extrapolated to zero organic modifier content.

INTRODUCTION

Studies of quantitative structure–activity and structure–toxicity relationships[1,2] have shown that the octanol–water partition coefficient (P) is one of the most important physical parameters related to the biological activities and toxicities of organic compounds. The shake-flask method[3,4] is usually used for the determination of log P. However, this method is tedious and not simple. Reversed-phase high-performance liquid chromatography (RP-HPLC) has also been used to determine log P, as it is simple, rapid and accurate. Octadecylsilica with[5,6] or without[7–11] previous treatment with trimethylsilyl chloride is the most widely used stationary phase.

The method involves:

(a) a linear correlation between capacity factor (log k') and organic modifier volume fraction (φ):

$$\log k' = \log k'_w + S\varphi \tag{1}$$

where

$$\varphi = V_{\text{Org. modifier}}/(V_{\text{Org. modifier}} + V_{\text{water}}) \tag{2}$$

the intercept (log k'_w) is the capacity factor extrapolated to zero organic modifier content and the slope, S, is the slope parameter[12];

(b) a linear regression between log k'_w and log P for several compounds (training

set) with known partition coefficients, usually determinated by the shake-flask method:

$$\log P = a + b \log k'_w \tag{3}$$

(c) the determination of the log k' and log k'_w values for the test compounds by chromatography; the log P values of the test compounds are obtained from eqn. 3.

In eqn. 3, partition conditions are represented by a value of b close to unity. However, a search for a chromatographic system giving a regression line in which a large change in log P corresponds to a small modification of log k' ($b > 1$) is especially important. A value of b of about 2 may be useful[1].

On the other hand, calculation methods[13–17] could be used to avoid the experimental determination of log P.

In connection with our work on non-ionic contrast agents, several 5-amino-2,4,6-triiodoisophthalic and 3,5-diamino-2,4,6-triiodobenzoic acid derivatives have been prepared. As part of a study of the physico-chemical properties of the contrast agents, we report here the determination of their chromatographic parameters log k'_w and S, and the relationship between log k'_w and calculated partition coefficients.

EXPERIMENTAL

Materials

HPLC-grade acetonitrile were obtained from Fluka (Buchs, Switzerland). Contrast agents 3–7 (Table I) were prepared by us[18,19]. Compounds 1 (iohexol) and 2 (iopamidol) were isolated from commercially available pharmaceutical products.

Chromatography

The HPLC instrument consisted of a Hewlett-Packard (Waldbronn Analytical Division, Waldbronn, F.R.G.) chromatograph with an autosampler and an

TABLE I

TRAINING SET OF CONTRAST AGENTS
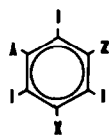
Compound	X	Y^a	Z^a
1	CONHCH$_2$CH(OH)CH$_2$OH	CONHCH$_2$CH(OH)CH$_2$OH	N(Ac)CH$_2$CH(OH)CH$_2$OH
2	CONHCH(CH$_2$OH)$_2$	CONHCH(CH$_2$OH)$_2$	NHCOCH(OH)CH$_3$ (L)^b
3	CONHCH$_2$CH(OH)CH$_2$OH	N(Ac)CH$_2$CH(OH)CH$_2$OH	N(Ac)CH$_2$CH(OH)CH$_2$OH
4	CONHCH$_2$CH(OH)CH$_2$OH	N(Ac)CH$_2$CH(OH)CH$_2$OH	N(Ac)CH$_3$
5	CONHC(CH$_2$OH)$_3$	N(Ac)CH$_2$CH(OH)CH$_2$OH	N(Ac)CH$_3$
6	CONHCH$_2$CH$_2$OH	N(Ac)CH$_2$CH(OH)CH$_2$OH	N(Ac)CH$_2$CH(OH)CH$_2$OH
7	CON(CH$_3$)CH$_2$CH$_2$OH	N(Ac)CH$_2$CH(OH)CH$_2$OH	N(Ac)CH$_2$CH(OH)CH$_2$OH

^{a} Ac = Acetyl.
^{b} (L) = Chiral center configuration of the lactoyl group.

HP 1090 A detection system, operating at 254 nm, HP 85 B computer, HP 9121 disc drive and HP Thinkjet printer.

A reversed-phase Novapack C_{18} column (15 cm × 3.7 mm I.D., 4 μm particle size) (Millipore–Waters, Milford, MA, U.S.A.) was used.

Retention times were measured by injecting 5 μl of an aqueous contrast agent solution (1 mg/ml) and eluting under isocratic conditions with several acetonitrile–water volume fractions (eqn. 2). The column temperature was 40°C in order to ensure adequate thermostating and good reproducibility of the chromatographic data. Two flow-rates, 0.5 and 1.0 ml/min, were used. The column dead time, t_0, was determined at each flow-rate and φ used by injecting 3% sodium nitrate solution as the non-retained compound. The capacity factor, k', is defined as

$$k' = (t_R - t_0)/t_0 \tag{4}$$

where t_R is the mean and weighted retention time of the test compound.

The experimental conditions were chosen in order to obtain short retention times (t_R) without losing the discrimination power between the different contrast agents. Hence broad chromatography peaks and thus inaccurate determinations of t_R can be avoided. Fortunately, owing to the high aqueous solubility of the contrast agents, it was possible to work with small t_R and φ values in order to obtain a linear correlation according to eqn. 1.

RESULTS AND DISCUSSION

Log P calculation

The Hansch–Leo method[14] is the most generally used procedure for the calculation of log P values. They suggested a group contribution method based on fragment f_i and corrective factor F_j values:

$$\log P = \sum_i a_i f_i + \sum_j b_j F_j \tag{5}$$

For molecules as complex as the contrast agents, with numerous inter- and intramolecular interactions, the calculated log P values deviated from the experimental results. This was evident when we used the experimental log P values obtained from Haavaldsen *et al.*[20] to evaluate the fit of this parameter using the Hansch–Leo procedure[14] (Table II), the log P values calculated by the Hansch–Leo method being much more positive than the experimental values. This does not mean that this method is invalid but implies that as we can not modify the group contributions f_i in eqn. 5, the F correction factors, especially the proximity factors (F_p), are overestimated and must be corrected.

The modifications made in this work in order to obtain a good correlation between the calculated and experimental values were as follows: (1) F_{p_3} proximity factors were not considered, except for X and/or Y = $CONHC(CH_2OH)_3$; (2) all F_{p_2} factors of the amido and carbamoyl groups with a hydroxyl moiety were considered, except for X and/or Y = $CONHCH(CH_2OH)_2$, where this contribution was divided

TABLE II

CALCULATED AND REPORTED LOG P VALUES

Compound	X	Y	Z
1	$CONHCH_2CH(OH)CH_2OH$	$CONHCH_2CH(OH)CH_2OH$	$N(Ac)CH_2CH(OH)CH_2OH$
8	$CON(CH_3)CH_2CH(OH)CH_2OH$	$CON(CH_3)CH_2CH(OH)CH_2OH$	$NHCOCH_3$
9	$CON(CH_3)CH_2CH(OH)CH_2OH$	$CON(CH_3)CH_2CH(OH)CH_2OH$	$N(Ac)CH_2CH_2OH$
10	$CONHCH(CH_2OH)_2$	$CONHCH(CH_2OH)_2$	$NHCOCH_3$
11	$CONHCH_2CH(OH)CH_2OH$	$CONHCH_2CH(OH)CH_2OH$	$N(Ac)CH_2CH_2OH$
12	$CONHCH_2CH_2OH$	$CONHCH_2CH_2OH$	$N(Ac)CH_2CH(OH)CH_2OH$
13	$CONHCH_2CH(OH)CH_2OH$	$CONHCH_2CH(OH)CH_2OH$	$N(Ac)CH_3$
14	$CONHCH(CH_2OH)_2$	$CONHCH(CH_2OH)_2$	$N(Ac)CH_2CH_2OH$
15	$CONHCH(CH_2OH)_2$	$CONHCH(CH_2OH)_2$	$N(Ac)CH_2CH(OH)CH_2OH$

[a] From ref. 20.
[b] Calculated according to the Hansch–Leo method.
[c] $\Delta^* = \log P_{obs} - \log P_{HL}$; $\Delta^{**} = \log P_{obs} - \log P_{CHL}$; $\Delta^{***} = \log P_{obs} - \log P_{calc}$.
[d] Calculated according to the corrected Hansch–Leo method.
[e] From eqn. 7.

by a factor of two; and (3) the hydroxyl–hydroxyl F_{p_2} factors were calculated from the following empirical equation, obtained by a trial and error procedure:

$$F'_{p_2}(OH, OH) = [(A - B)C/N]F_{p_2}(OH, OH) \qquad (6)$$

where A is the number of chains with two or more OH groups, B is the number of chains with less than two OH groups, C is the number of chains with OH and N is the total number of hydroxyl groups.

We modified the contribution of the $F_{p_2}(OH, OH)$ factors to consider both the number and the molecular distribution of the OH groups. Hence there are two factors in eqn. 6: (a) C/N can unmodify or decrease the magnitude of the $F_{p_2}(OH, OH)$; and (b) $(A - B)$ can unmodify, increase or decrease the magnitude of the $F_{p_2}(OH, OH)$ and even reverse the sign of this contribution $(A < B)$, which is always positive in the Hansch–Leo method.

The following equation shows a good correlation between log P calculated as above and reported experimental values[20]:

$$\log P_{obs} = 0.067 + 1.053 \log P_{CHL} \qquad (7)$$

$$n = 9; \ r = 0.966; \ SEE = 0.101; \ F(1,7) = 97.82; \ p < 0.0001.$$

where SEE = standard error of estimation; n = number of data points (compounds); r = correlation coefficient; F = F-statistic significance test with 1 and 7 degrees of freedom; p = observed significance level of F (probability).

Log P determination

Table III gives the capacity factors at different organic modifier volume fractions (log k'_φ) obtained with flow-rates of 0.5 and 1 ml/min. In the latter instance,

Log $P_{obs}{}^a$	Log $P_{HL}{}^b$	Δ^{*c}	Log $P_{CHL}{}^d$	Δ^{**c}	Log $P_{calc}{}^e$	Δ^{***c}
−3.05	−1.71	−1.34	−2.99	−0.06	−3.08	0.03
−2.17	−0.82	−1.35	−2.10	−0.07	−2.14	−0.03
−2.28	−1.18	−1.10	−2.28	0.00	−2.33	0.05
−2.27	0.10	−2.37	−2.17	−0.10	−2.22	−0.05
−2.47	−1.24	−1.23	−2.34	−0.13	−2.40	−0.07
−1.86	−0.32	−1.54	−1.81	−0.05	−1.84	−0.02
−2.05	−0.81	−1.24	−2.09	0.04	−2.13	0.08
−2.33	−0.25	−2.08	−2.43	0.10	−2.49	0.16
−2.80	−0.73	−2.07	−2.57	−0.23	−2.64	−0.16

the acetonitrile concentration can be decreased to 5% ($\varphi = 0.05$) without increasing the retention times too much.

Fig. 1 shows the linear correlations of φ with log k' obtained at a flow-rate of 0.5 ml/min for compounds 1–7. Table IV gives the linear regression data for the correlations and also the log P values calculated by the corrected Hansch–Leo method. The intercept log k'_w shows the degree of affinity of the compound for the lipophilic phase when aqueous elution occurs. The slope S shows the reduction in the affinity of the compound for the stationary phase with increase in the organic modifier concentration.

The relationship between log k'_w and calculated partition coefficients, log P_{CHL}, is expressed by the following equations:

$$\log P_{CHL} = -2.113 + 1.813 \log k'_w \tag{8}$$

$$n = 7; \ r^* = 0.980; \ SEE = 0.221; \ F(1,5) = 123.38; \ p < 0.001$$

and

$$\log P_{CHL} = -2.244 + 2.007 \log k'_w \tag{9}$$

$$n = 6; \ r = 0.998; \ SEE = 0.072; \ F(1,4) = 1098.55; \ p < 0.001.$$

The data referring to these equations are given in Table IV. Eqn. 9 is obtained from the same data as eqn. 8, excluding the most deviating point (residual = 0.384) corresponding to iopamidol. It is noteworthy that there is an improvement in the quality of the regression on going from eqn. 8 to 9.

The "deviant" behaviour of iopamidol could be explained by its structural dissimilarities with the other compounds in the training set. Moreover, the calculated log k'_w values for iohexol (-0.367) and iopamidol (-0.366) were almost identical and

TABLE III

RP-HPLC CAPACITY FACTORS (LOG k'_φ) OF CONTRAST AGENTS

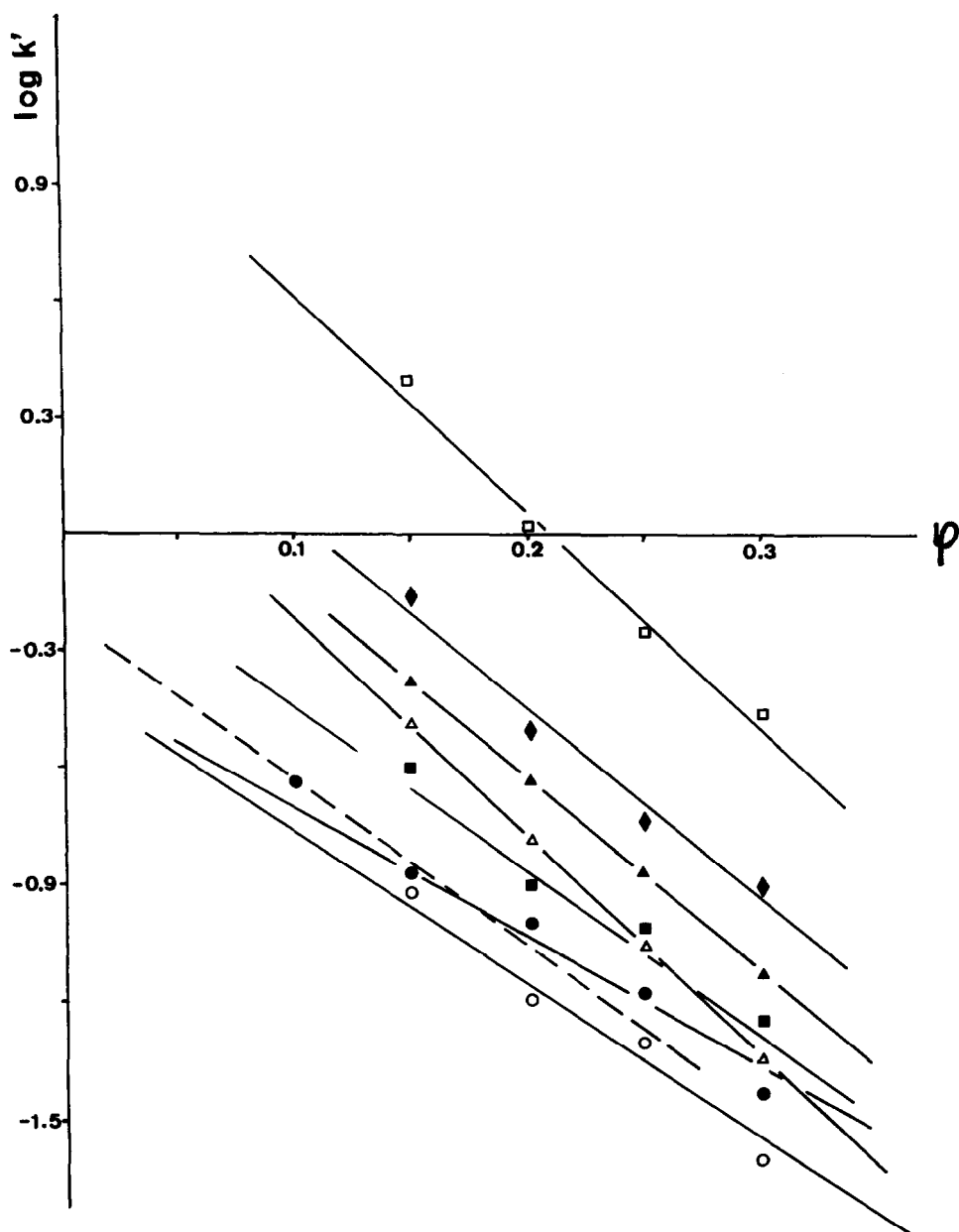| Compound	Flow-rate 0.5 ml/min				Flow-rate 1.0 ml/min				
	$Log\ k'_{0.15}$	$Log\ k'_{0.20}$	$Log\ k'_{0.25}$	$Log\ k'_{0.30}$	$Log\ k'_{0.05}$	$Log\ k'_{0.10}$	$Log\ k'_{0.15}$	$Log\ k'_{0.20}$	$Log\ k'_{0.25}$
1	−0.887	−1.100	−1.196	−1.439	−1.221	−1.561	−1.309	−1.201	−1.291
2	−0.917	−1.194	−1.303	−1.524	−0.983	−1.505	−1.353	−1.318	−1.439
3	−0.600	−0.906	−1.009	−1.243	−0.373	−1.196	−1.077	−1.055	−1.047
4	−0.164	−0.509	−0.732	−0.910	0.058	−0.596	−0.484	−0.613	−0.732
5	0.384	0.042	−0.242	−0.460	0.699	0.015	0.096	−0.016	−0.250
6	−0.478	−0.788	−1.044	−1.336	−0.437	−0.974	−0.814	−0.838	−1.064
7	−0.383	−0.647	−0.861	−1.149	−0.166	−0.796	−0.729	−0.824	−0.884

Fig. 1. Relationship between log k' values of CA and acetonitrile concentration (φ) in the mobile phase (flow-rate 0.5 ml/min). The compounds are numbered as in Table I. Key: $\bigcirc$ = 1; $\bullet$ = 2; $\blacksquare$ = 3; $\blacklozenge$ = 4; $\square$ = 5; $\triangle$ = 6; $\blacktriangle$ = 7.

hence the partition coefficients calculated by eqn. 8 and 9 were also the same. This result conflicts with the experimental data found by Haavaldsen et al.[20] for iohexol (log P = −3.046) and by Jacobsen[21] for the iohexol (log P = −3.000) and iopamidol
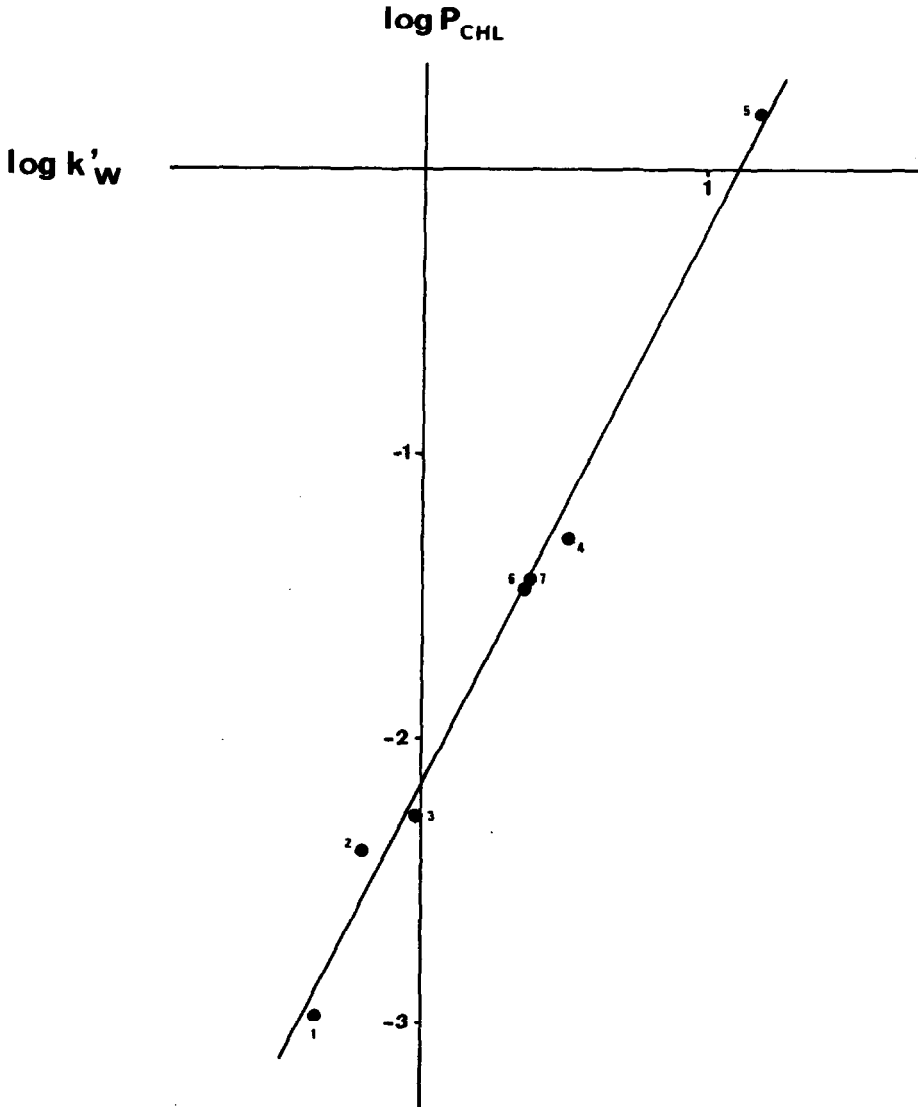
Fig. 2. Correlation of calculated log P_{CHL} with log k'_w. Regression data are given in eqn. 10.

(log P = −2.699). To obviate this discrepancy, log k'_w of iopamidol was recalculated using three data points at φ = 0.10, 0.15 and 0.20, giving log k'_w = −0.208. By including this new log k'_w value, the following equation is obtained (Fig. 2):

$$\log P_{CHL} = -2.185 + 1.931 \log k'_w \tag{10}$$

$$n = 7; \, r = 0.994; \, SEE = 0.120; \, F(1,5) = 428.67; \, p < 0.001.$$

TABLE IV

LOG P_{CHL} VALUES OF THE TRAINING SET AND LINEAR REGRESSION DATA FOR THE CORRELATIONS OF LOG k' AND φ

Compound	Log P_{CHL}	Calculated for log $k' = \log k'_w + S\varphi$					
		Log k'_w	S	r	SEE^a	F(1,2)	n
1	−2.988[b]	−0.367	−3.504	−0.988	0.043	82.74[d]	4
2	−2.393[c]	−0.366	−3.860	−0.988	0.048	79.19[d]	4
3	−2.267	−0.025	−4.064	−0.984	0.058	60.07[d]	4
4	−1.308	0.529	−4.922	−0.988	0.060	83.32[d]	4
5	0.191	1.198	−5.632	−0.995	0.044	206.12[d]	4
6	−1.484	0.362	−5.660	−0.999	0.016	1647.92[e]	4
7	−1.457	0.370	−5.024	−0.999	0.021	691.30[e]	4

[a] SEE = standard error of estimate.
[b] Reported values: −3.046[20]; −3.000[21].
[c] Reported value: −2.699[21].
[d] $p \leqslant 0.01$.
[e] $p \leqslant 0.001$.

Log P values of compounds 1–7 obtained using eqn. 10 are −2.894 (1), −2.587 (2), −2.233 (3), −1.164 (4), +0.128 (5), −1.486 (6) and −1.471 (7). As can be seen, log P for iohexol and iopamidol calculated by the corrected Hansch–Leo method (Table IV) and by eqn. 10 are close to the literature reported values (Table IV).

On the other hand, as can be seen in Table III, the log k' values obtained at a flow-rate of 1 ml/min were poorly correlated with φ. This shows that with highly water-soluble compounds such as the contrast agents, an increase in flow-rate may cause a deviation of the partition phenomenon, probably owing to the poor retention of the compounds under these conditions.

CONCLUSION

The measurement of partition coefficients of highly water-soluble contrast agents by RP-HPLC is a viable alternative to the tedious shake-flask method. It is possible to calculate log P values of molecules as complex as the contrast agents by making slight modifications to the Hansch–Leo method. The calculated log P values showed a high correlation with experimental log P values (eqn. 7) and with log k'_w (eqns. 9 and 10). As Leo reported[22], the deviation between calculated log P and values determined by RP-HPLC may be due more to unsuitable experimental conditions than to a formal error in the calculation procedure.

Once it has been verified that the method used for the calculation of log P affords accurate results for a set of compounds, the use of calculated log P values correlated with log k'_w values for the training set allows experimental log P values for compounds not included in that set to be obtained.

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