Determination of the Partition Coefficient and Acid Dissociation Constants of Iodochlorhydroxyquin by an Improved Partition Method

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Abstract □ A simple and precise method for determining partition coefficients was developed utilizing a disposable injector. The method was used to determine the partition ratio of iodochlorhydroxyquin between *n*-decane and phosphate-buffered saline, pH 7.4. The partition ratio was found to be 1750 with a coefficient of variation of 3%. Moreover, it was found that iodochlorhydroxyquin did not associate in *n*-decane at a concentration $< 1 \times 10^{-3}$ M. The acid dissociation constants and the partition coefficient of the undissociated species were determined. These values corresponded well with the values obtained from spectrophotometric methods.

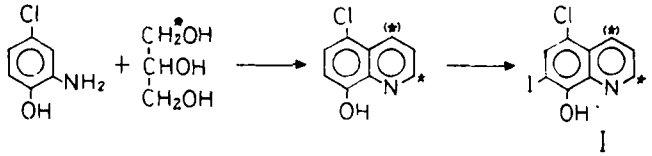
Keyphrases □ Iodochlorhydroxyquin—partition coefficients, acid dissociation constants □ Improved partition method—partition coefficients, dissociation constants, iodochlorhydroxyquin

Iodochlorhydroxyquin (5-chloro-7-iodo-8-quinolinol; I), an amphoteric molecule sparingly water soluble at neutral or slightly acidic pH values, has been studied *in vivo* using a radionuclide-labeled compound (1). Repeated administrations of I to beagle dogs led to neurological disorders with concomitant demonstration of I in the central nervous system (CNS) by quantitative GC measurements (2). Intestinal absorption, renal excretion, and the distribution of I to various tissues, especially to the CNS, were thought to be influenced by the concentration of the undissociated species. The acid dissociation constants and partition coefficient of the undissociated species have not been reported, primarily because I is extremely insoluble in water and is strongly adsorbed onto glassware.

Special glassware was used to determine the partition ratio of long-chain fatty acids between *n*-heptane and an aqueous phosphate buffer (3); however, accurate values >1000 were not determined. Using a disposable injector, we have developed a more precise and convenient method for determining large partition ratios. This methodology has been used to determine the partition coefficient of the undissociated species and acid dissociation constants of I.

EXPERIMENTAL SECTION

Syntheses of Tritiated Analogues of 5-Chloro-7-iodo-8-quinolinol—Tritiated I was synthesized according to the method reported by Urakubo and Kido (4) (Scheme I). To a mixture of 2-amino-4-chlorophenol[1] (14.4 mg, 0.1 mmol), 4-chloro-2-nitrophenol[1] (8.8 mg, 0.05 mmol), glycerol[2] (0.11 mmol), and [1(3)-^{3}H]glycerol[3] (1.6 mCi, 2.3 Ci/mmol) was added 21 μL of concentrated sulfuric acid. The mixture was refluxed at 125°C for 2 h, and then the remaining 4-chloro-2-nitrophenol was removed by sublimation (the temperature was increased to 160°C for 15 min). The residual material was extracted twice with 1.5 mL of distilled water and with the same volume of 7% H_2SO_4. The total aqueous phase was adjusted to pH 5 with 30% NaOH, and the resulting precipitate was removed by centrifugation and washed with distilled water to give 5-chloro-8-[2(4)-^{3}H]quinolinol as a gray powder, which was purified by sublimation at 140°C. This afforded 10.7 mg (60% yield) of crystalline material, mp 129°C (a radiochemical yield of 26%). 8-[2(4)-^{3}H]Quinolinol was synthesized in a similar manner (30% yield), mp 76°C.



Scheme I

Preparation of 5-Chloro-7-iodo-8-[2(4)-^{3}H]quinolinol—5-Chloro-8-[2(4)-^{3}H]quinolinol (8.6 mg) was dissolved in 3 mL of 60 mM glycine buffer (pH 1.35) and was iodinated with iodine monochloride as described by Hales and Randle (5). The excess reagent was decomposed by adding 0.5 M $Na_2S_2O_3$, the precipitate was washed twice with the buffer, twice with distilled water, and was then dried under reduced pressure over phosphorus pentoxide to give 12.1 mg (83% yield) of 5-chloro-7-iodo-8-[2-(4)-^{3}H]quinolinol as a white solid, mp 173°C (dec.).

The chromatographic behavior (TLC, silica gel) and the melting points of these labeled compounds were identical with those of authentic cold materials (6). The radiochemical purity of tritiated I was determined to be 99.3% by an isotopic recrystallization method.

Other Chemicals—*n*-Decane[1], reagent grade, was purified by shaking several times with concentrated sulfuric acid until the acidic layer became colorless, and was then distilled under reduced pressure. The buffers were 0.02 M, containing 0.13 M NaCl and 5 mM EDTA to prevent the interference of metal ions; pH values of the buffer were adjusted with an accuracy of ±0.01. The scintillation cocktail consisted of 5 g of 2,5-diphenyloxazole (PPO)[4] and 134 mg of 2,2′-*p*-phenylene-*bis*(4-methyl-5-phenyloxazole) (dimethyl POPOP)[5] per liter of toluene[2]. All other chemicals were of the highest purity available.

The General Procedure for the Measurement of Partition Ratios—A glass tube (19 mm i.d. × 37 mm depth) containing 3.5 mL of buffer was covered with a polyethylene cap with two holes (4 mm i.d. and 1 mm i.d., respectively). A 25-gauge disposable needle[6] was inserted into the tube through the larger

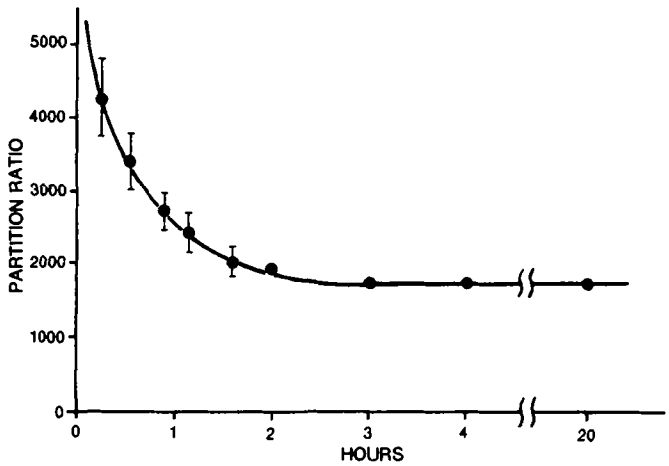


Figure 1—*Time course of partition equilibrium of iodochlorhydroxyquin between n-decane and 0.02 M phosphate-0.13 M NaCl-5 mM EDTA buffer (pH 7.4) at 25°C. [^{3}H]Iodochlorhydroxyquin was initially added to the organic layer.*

[1] Tokyo Kasei, Tokyo.
[2] Kanto Kagaku, Tokyo.
[3] Ethanol solution; Amersham International, Amersham, Buckinghamshire HP 79LL, England.
[4] Nakarai Kagaku, Kyoto.
[5] Eastman Kodak, Rochester, N.Y.
[6] Terumo, Tokyo.

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Table I—Determination of the Dissociation Constants of Iodochlorhydroxyquin at 25°C

In Dilute Hydrochloric Acid					
HCl, M	pH	$\frac{A_{266}}{A_{252.5}}$	$\log \frac{[CF^+]}{[CF]}$	pK_1'	$pK_{1,0}$[a]
0.0117	2.02	1.102	0.180	2.20	2.15
0.0092	2.12	1.100	0.176	2.30	2.25
0.0075	2.21	1.026	0.035	2.25	2.20
0.0057	2.32	0.961	−0.078	2.24	2.20
0.0048	2.39	1.000	−0.011	2.38	2.34
0.0039	2.48	0.913	−0.159	2.32	2.29
0.0028	2.61	0.824	−0.306	2.30	2.28
0.0023	2.70	0.794	−0.357	2.34	2.32
Mean ± *SD*					2.25 ± 0.07
In 1 mM Sodium Carbonate Buffer					
	pH	$\frac{A_{266}}{A_{252.5}}$	$\log \frac{[CF]}{[CF^-]}$	pK_2'	$pK_{2,0}$[b]
	8.10	1.164	0.208	8.31	8.33
	8.20	1.306	0.055	8.26	8.28
	8.29	1.375	−0.022	8.27	8.29
	8.40	1.463	−0.125	8.28	8.30
	8.41	1.426	−0.081	8.33	8.35
	8.50	1.540	−0.224	8.28	8.30
	8.58	1.554	−0.243	8.34	8.36
	8.70	1.594	−0.299	8.40	8.43
Mean ± *SD*					8.33 ± 0.05

[a] $pK_{1,0}$ was calculated from Eq. 9. [b] $pK_{2,0}$ was calculated from Eq. 10.

hole of the cap and fixed so that the tip of the needle almost reached the bottom. A disposable tuberculine syringe (2.5 mL)[6] was connected to the needle. The *n*-decane solution of [^{3}H]iodochlorhydroxyquin (0.2 mL) was gently placed on the aqueous layer with a microsyringe through the smaller hole of the cap. The tubes were gently shaken by a reciprocating shaker[7] 73 times per min for 3 h at 25 ± 0.5°C. After the equilibration, 2 mL of the aqueous layer was removed (with the tuberculine syringe) and the solution was transferred to a weighed scintillation vial. The vial was weighed again to accurately determine the volume of the sample. After removal of the polyethylene cap with the needle, 50 μL of the organic layer was removed and transferred to a weighed scintillation vial. The volume was again determined by weighing the vial. Ten milliliters of the scintillation cocktail was added to each vial and the radioactivity was counted with a liquid scintillation counter[8]. The vial containing the aqueous sample was shaken 100 times before counting.

RESULTS AND DISCUSSION

Determination of the Partition Ratio of I by the Partition Method—The partition ratio is defined as follows:

$$\text{Partition ratio} = \frac{\text{Radioactivity in organic phase}}{\text{Radioactivity in aqueous phase}} \qquad \text{(Eq. 1)}$$

Figure 1 shows that the partition equilibrium was reached after 3 h and was maintained for ≥17 h with a variation of ±3%. As shown in Fig. 2, the partition ratios of I, 8-quinolinol, and 5-chloro-8-quinolinol at pH 7.4 were measured over a wide concentration range. Since the partition ratios remained constant, it appeared that the 8-quinolinols did not associate in a manner similar to the fatty acids (3). The partition ratio of I was 1750, while those of 8-quinolinol and 5-chloro-8-quinolinol were 24.1 and 247, respectively.

Determination of the Dissociation Constants of I by the Partition Method—The partition ratio (Eq. 2) assumes that the ionized forms of I are not found in the organic phase and that un-ionized molecules of I are found in both phases.

$$\text{Partition ratio} = \frac{[CF]_o}{[CF^+]_w + [CF]_w + [CF^-]_w} = \frac{K_p}{\frac{(H^+)_w}{K_1'} + 1 + \frac{K_2'}{(H^+)_w}} = \frac{K_p}{10^{(pK_1'-pH)} + 1 + 10^{(pH-pK_2')}} \qquad \text{(Eq. 2)}$$

[7] BT-21; Yamato Kagaku, Tokyo.
[8] Tri-Carb Model 3255; Packard Instruments, Downers Grove, Ill.

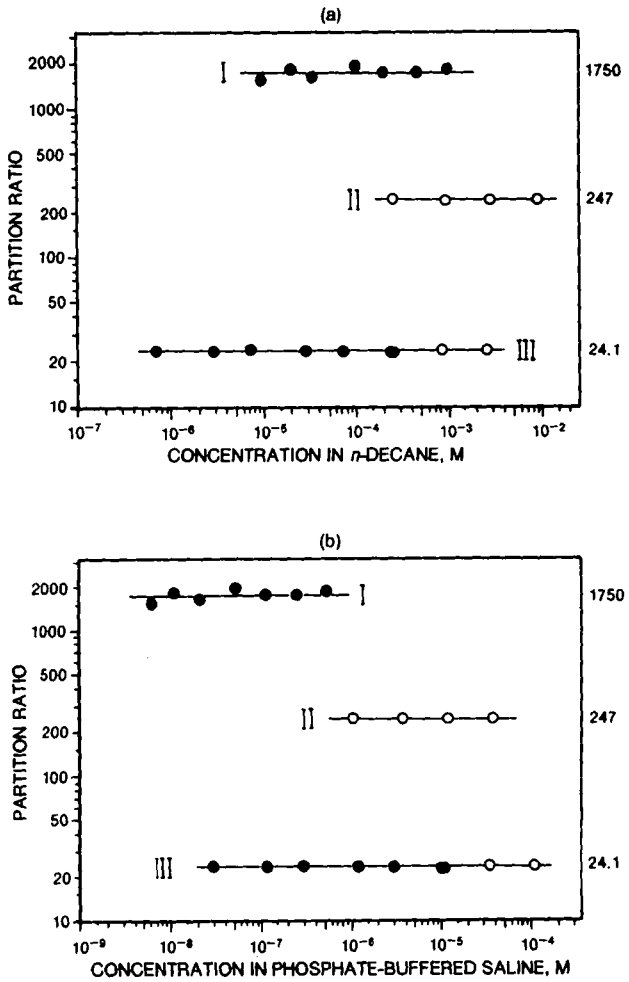

Figure 2—*Effect of concentration on the partition ratio of iodochlorhydroxyquin (I), 5-chloro-8-quinolinol (II), and 8-quinolinol (III) between n-decane and phosphate-buffered saline (pH 7.4) at 25°C. Key: (●) radioisotope method; (○) values from the spectrophotometric measurement.*

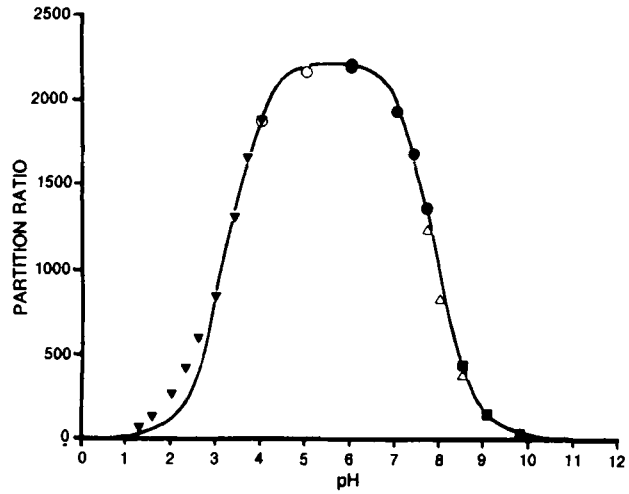

Figure 3—*Effect of pH on the partition ratio of iodochlorhydroxyquin between n-decane and the 0.02 M buffer–0.13 M NaCl–5 mM EDTA system at 25°C. Key: (▼) glycine–hydrochloric acid; (○) acetate; (●) phosphate; (△) Tris-HCl and (■) glycine–sodium hydroxide; (—) hypothetical curve for Eq. 3.*

In Eq. 2, the subscripts o and w indicate the organic and the aqueous phases, respectively. [CF], [CF$^+$], [CF$^-$], and (H$^+$) represent the concentration of the molecular, the cationic, and the anionic forms of I, and the activity of the hydrogen ion, respectively. The partition coefficient, K_p, is expressed as $K_p = [CF]_o/[CF]_w$. The dissociation constants of I in acidic and basic media are expressed as $K_1' = [CF]_w(H^+)/[CF^+]_w$, and $K_2' = [CF^-]_w(H^+)/[CF]_w$, respectively. Accordingly, those constants may be calculated from the pH profile of the partition ratio.

The partition ratio at various pH values (1.2–9.8) was measured using five buffer systems. Figure 3 shows that the pH profile of the partition ratios in I was a bell-shaped curve with a maximum at pH 5.6. The constants, K_p, K_1', and K_2' were estimated from the data in Fig. 3 as follows: $K_p = 2230 \pm 43$ (CV 1.9%), $n = 11$; $K_1' = 5.6 \times 10^{-4}$, $pK_1' = 3.25$; $K_2' = 1.29 \times 10^{-8}$, $pK_2' = 7.89$. Consequently, Eq. 2 can be expressed as:

$$\text{Partition ratio} = \frac{2230}{10^{(3.25-pH)} + 1 + 10^{(pH-7.89)}} \qquad \text{(Eq. 3)}$$

The solid line in Fig. 3 is the hypothetical curve for Eq. 3, and it agrees well with the observed values in the pH range of 3–10.

Inquiry of the Partition Method by a Spectrophotometric Approach—Compound I and its ionized forms show characteristic UV absorption. The dissociation constants of such compounds, in general, can be calculated from the spectral data. The spectrophotometric method is based on the general equation:

$$A = [CF^+]\epsilon^c + [CF]\epsilon^n + [CF^-]\epsilon^a \qquad \text{(Eq. 4)}$$

where A, total absorbance, is the sum of the respective absorbance of each species, and ϵ^n, ϵ^c, and ϵ^a represent the molar extinction coefficients of the un-ionized, cationic, and anionic forms of I, respectively. When the pH value is lower than $pK_2' - 2$ or higher than $pK_1' + 2$, the term [CF$^-$] or [CF$^+$] can be neglected, respectively. In this case, pK_1' and pK_2' are as follows:

$$pK_1' = pH + \log\frac{[CF^+]}{[CF]} \qquad \text{(Eq. 5)}$$

$$pK_2' = pH + \log\frac{[CF]}{[CF^-]} \qquad \text{(Eq. 6)}$$

Both the anionic and cationic forms of I showed an absorption maximum at 266 nm, and the values of ϵ_{266}^a or ϵ_{266}^c, calculated from the spectrophotometric data by dissolving I in 1 mM sodium carbonate buffer or in diluted hydrochloric acid, were 3.81×10^4 and 2.80×10^4, respectively. However, the value of ϵ^n could not be determined because I was sparingly soluble in the neutral aqueous solution. Accordingly, we estimated $\epsilon_{\lambda_{max}}^n$ (252.5 nm) and ϵ_{266}^n as 1.2×10^4 and 0.42×10^4, respectively, by extrapolating the ϵ values measured in the various concentrations of methanol (Fig. 4). $\epsilon_{252.5}^c$ and $\epsilon_{252.5}^a$ were found to be 2.00×10^4 and 1.92×10^4, respectively. Thus, Eqs. 5 and 6 may be expressed as follows:

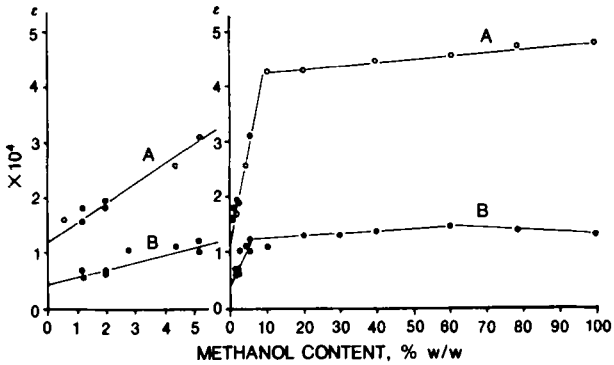

Figure 4—*Effect of methanol content in aqueous solution on the ε of iodochlorhydroxyquin at 252.5 (A) and 266(B) nm.*

$$pK_1' = pH + \frac{1.20 \times A_{266}/A_{252.5} - 0.42}{2.80 - 2.00 \times A_{266}/A_{252.5}} \qquad \text{(Eq. 7)}$$

$$pK_2' = pH + \frac{1.92 \times A_{266}/A_{252.5} - 3.81}{0.42 - 1.20 \times A_{266}/A_{252.5}} \qquad \text{(Eq. 8)}$$

The dissociation constants of I were calculated from the data listed in Table I. Similarly $pK_{1,0}$ and $pK_{2,0}$, calculated from the following Debye-Hückel equations, were 2.25 and 8.33 at 25°C, respectively.

$$pK_1' = pK_{1,0} + 0.509\sqrt{I} \qquad \text{(Eq. 9)}$$

$$pK_2' = pK_{2,0} - 0.509\sqrt{I} \qquad \text{(Eq. 10)}$$

where I represents the ionic strength.

The values pK_1' and pK_2', obtained from the spectrophotometric results at the same temperature and the same ionic strength as the partition method, were 3.17 ± 0.11 and 8.05 ± 0.08 at 25°C, respectively, and agree well with those obtained by the partition method.

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