## 325. *Lipoid–Water Partition Coefficients of Some Aromatic Bases.*

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The partition coefficients between liquid paraffin and aqueous phosphate buffer (pH 7·2) were determined by a spectrophotometric method for 26 aromatic bases exhibiting tuberculostatic activity.

ONE of the factors influencing the permeability of a cell membrane to a drug molecule must be the lipoid–water partition coefficient of the drug. In order to discover the effect of variation in partition coefficient on antituberculous activity, this constant has been determined for a number of aromatic amines. The substances were bases of $pK_a$ values ranging from 3 to 10, and the two phases were liquid paraffin and an aqueous buffer solution of pH 7·2 (the physiological pH).

Albert, Goldacre, and Heymann (*J.*, 1943, 651) and Hammick and Mason (*J.*, 1950, 348) used a colorimetric method for the determination of the partition coefficients of acridines, whereas Golumbic *et al.* (*J. Amer. Chem. Soc.*, 1949, **71**, 2624; 1950, **72**, 4145; 1951, **73**, 3966) employed a spectrophotometric technique for phenols and amines, estimating the concentration of organic material in the lipoid phase. In the present work it was found more convenient to use spectrophotometric estimation of the organic base in the aqueous phase.

The partition coefficient $k$ of a base distributed between two immiscible liquids is given by $k = [B]_0/[B]_w$, where $[B]_0$ and $[B]_w$ are the concentrations of the undissociated base in the two phases. The base in the aqueous phase dissociates according to $[B]_w[H^+]/[BH^+] = K_a$, where $K_a$ is the acid dissociation constant of the base. The apparent partition constant $k'$, which is actually measured, is given by $k' = [B]_0/([B]_w + [BH^+])$. At constant hydrogen-ion concentration the degree of dissociation of the base is constant and independent of the concentration. The relation between the apparent and the true partition coefficient then becomes $k/k' = 1 + [H^+]/K_a$, *i.e.*, the apparent partition coefficient is constant at constant pH and proportional to the true value of $k$. A similar derivation has been used by Craig (*J. Biol. Chem.*, 1943, **150**, 33).

If $pK_a$ of the base is known, the true value of $k$ can readily be calculated from $k/k' = 1 +$ antilog ($pK_a - $ pH). The ratio $k/k'$ can be obtained from the figure, which indicates that a linear relation is obtained when the unity term in the last equation is
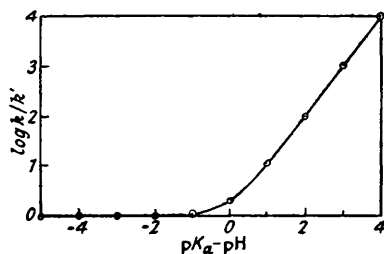


negligible in comparison with the antilog term, *i.e.*, when the base is substantially ionised. Such linear relation have been obtained by Hammick and Mason (*loc. cit.*) and Golumbic *et al.* (*loc. cit.*).

*Method.*—Dilute solutions of the salts were prepared by dissolving the pure hydrochloride of the base in M/500-phosphate buffer solution of pH 7·2 (Clark, " The Determination of Hydrogen Ions," Baillière, Tindall, and Cox, London, 1928, p. 210), the concentrations (*ca.* 1—10 mg. per 100 c.c. of soluton) being such as to give an optical density of 0·4—0·9 at the ultra-violet absorption maximum. The pH of the solution was checked on a Leeds and Northrup Universal pH meter. Known volumes of the hydrochloride buffer solution $(v_w)$ and of liquid paraffin (B.P., 1932) $(v_o)$ were equilibrated by mechanical shaking for 3 hours; this was sufficient for complete equilibration. After separation and centrifugation, the optical density of the aqueous layer was redetermined at the wave-length of maximum absorption which was always unchanged. The optical readings were taken on a Beckman quartz spectrophotometer, model DU, and the experiments were carried out at 20° ± 2°. If $d_1$ and $d_2$ are the initial and final maximum optical densities of the aqueous solutions, then (on the assumption that all the compounds investigated obey Beer's law), $k'$ is given by $k' = v_w(d_1 - d_2)/v_0d_2$.

The maximum optical densities were measured at the nearest millimicron and the slit width kept constant for the initial and final solution. The volumes of paraffin and water were chosen to give a final maximum optical density in the aqueous phase of 0·15—0·6; the ratio 1 : 1 was, however, never exceeded as larger volumes of paraffin made equilibration difficult. Duplicate readings at different concentrations agreed within 5%. At the low concentrations used, the possibility of molecular association in the paraffin layer was negligible; this assumption was justified by the constancy of the values obtained for $k'$ over a range of concentrations.

*Results and Discussion.*—The results, together with the ultra-violet absorption maxima, are given in Tables 1, 2, and 3. Table 1 comprises primary aromatic amines, all weakly basic with $pK_a$ <7, and substantially un-ionised at pH 7·2. Lipophilic character is seen to increase with molecular complexity, as expected. The abnormally low value of $k'$ for compounds 1 and 4 is probably caused by the heterocyclic substituent. Table 3 contains heterocyclic bases, all with $pK_a$ >7 and therefore appreciably ionised at pH 7·2. This is indicated by the low values for $k'$ for compounds similar in size to those in Table 1. The degree of dissociation, however, is by no means the only factor determining the magnitude

of $k'$. Thus, although the introduction of a methyl group into a base has a base-strengthening effect, compound 22 has a higher partition coefficient than no. 21. The same applies to compounds 24, 25, and 26, where $k'$ again increases with increasing numbers of methyl substituents and hence increasing $pK_a$. This abnormal behaviour of the methyl homologues is probably due to the solubilising properties of the methyl group causing an increase in $k'$ greater than the decrease occasioned by the inductive $(-I)$ effect of the alkyl group; this behaviour may be similar in nature to the effect of methyl substituents on the ionis-

TABLE 1.

| No. | Hydrochloride of | $k'$ | $\lambda_{max.}$ (m$\mu$) | No. | Hydrochloride of | $k'$ | $\lambda_{max.}$ (m$\mu$) |
|---|---|---|---|---|---|---|---|
| 1 | p-2-Pyridylaniline [1] ... | 0·47 | 293 | 7 | β-Naphthylamine ...... | 9·5 | 234 |
| 2 | o-Aminophenol [2] ......... | <1 | 229 | 8 | α-Naphthylamine ...... | 9·7 | 237 |
| 3 | p-Aminophenol [2] ......... | <1 | 231 | 9 | 2-Aminofluorene [4] ...... | 57 | 280 |
| 4 | p-Piperidinoaniline [3] ... | 1·28 | 242 | 10 | 9-Aminophenanthrene [5] | 72 | 250 |
| 5 | p-Toluidine .............. | 2·00 | 233 | 11 | 2-Aminochrysene [6] ...... | — | — |
| 6 | p-Chloroaniline ......... | 3·14 | 239 | | | | |

[1] Dihydrochloride, m. p. 310—313° (decomp.). Forsyth and Pyman (*J.*, 1926, 2912) give m. p. > 310°. [2] Approx. only, as the compound showed pronounced oxidation in the aqueous phase. [3] Dihydrochloride (Bauer and Cymerman, *J.*, 1950, 2078). [4] Plates (from methanol), m. p. 321—322° (decomp ) (Found : N, 6·8. $C_{13}H_{11}N$,HCl requires N, 6·45%). This salt rapidly lost hydrogen chloride when heated *in vacuo*. [5] M. p. 278—280° (decomp.). Schmidt and Strobel (*Ber.*, 1901, **34**, 1461) give m. p. 275° (decomp.). [6] Plates (from methanol), m. p. 274—275° (decomp.) (Found : N, 5·3. $C_{18}H_{13}N$,HCl requires, N, 5·0%). Measurement of $k'$ impossible owing to insolubility in water.

TABLE 2.

| No. | Compound | $k'$ | $\lambda_{max.}$ (m$\mu$) |
|---|---|---|---|
| 12 | N'-4-Diphenylyl-NN-diethylethylenediammonium dichloride [1] .............. | 44·7 | 282 |
| 13 | N'-4-Diphenylyl-NN-diethyl-NN'N'-trimethylethylenediammonium di-iodide ................................................................ | 0·065 | 225 |
| 14 | N-4'-Diphenylyl-2-morpholinoethylammonium dichloride [1] ................. | 74·9 | 285 |
| 15 | N-4'-Diphenylyl-N-methyl-2-morpholinoethylamine dimethiodide [1] ........ | 0·16 | 224 |
| 16 | 4-2''-Diethylaminoethoxydiphenyl hydrochloride [1] ........................... | 104 | 257 |
| 17 | 4-2''-Diethylaminoethoxydiphenyl methiodide [1] .............................. | 0·12 | 259 |
| 18 | N-4-Diphenylyltrimethylammonium iodide [2] ................................. | 0·04 | 228 |
| 19 | 4-Benzylamino-4'-dimethylaminodiphenyl dihydrochloride [1] ................ | 45·0 | 273 |

[1] Bauer, Cymerman, and Sheldon, *J.*, 1951, 2342.   [2] Vorlander, *Ber.*, 1925, **58**, 1893.

TABLE 3.

| No. | Hydrochloride of | M. p. | Formula | Found : N (%) | Required : N (%) | $k'$ | $\lambda_{max.}$ (m$\mu$) |
|---|---|---|---|---|---|---|---|
| 20 | 4-Aminoquinoline [1] .............. | 308° | $C_9H_8N_2$,HCl | 15·5 | 15·5 | 0·02 | 230 |
| 21 | 5-Aminoacridine ................. | — | — | — | — | 0·15 | 259 |
| 22 | 5-Amino-1-methylacridine ...... | — | — | — | — | 0·19 | 260 |
| 23 | 5-Amino-1 : 2-benzacridine [2] ... | 380 (dec.) | $C_{17}H_{12}N_2$,HCl | 10·05 | 10·0 | 4·90 | 286 |
| 24 | 9-Aminophenanthridine ......... | 309 | $C_{13}H_{10}N_2$,HCl | 12·3 | 12·15 | 3·10 | 240 |
| 25 | 9-Amino-3-methylphenanthridine | 349—350 | $C_{14}H_{12}N_2$,HCl | 11·8 | 11·4 | 8·30 | 241 |
| 26 | 9-Amino-1 : 3-dimethylphenanthridine ......................... | 372 (dec.) | $C_{15}H_{14}N_2$,HCl | 11·2 | 10·8 | 47·0 | 242 |

[1] Plates (from methanol), anhyd. after drying at 120°. In air, it formed a *monohydrate* (Found : N, 14·2. $C_9H_8N_2$,HCl,$H_2O$ requires N, 14·15%).   [2] Pale yellow plates from methanol.

ation and water-solubilities of p-aminobenzenesulphonamidopyrimidines (Gilligan and Plummer, *Proc. Soc. Exp. Biol. Med.*, 1943, **53**, 142; Albert, " Selective Toxicity," Methuen, London, 1951, p. 36).

Table 2 shows the large (approximately thousand-fold) drop in $k'$ which occurs on going from a base to the methiodide in these compounds.

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