(1.8 times) if the buffered sample solution was allowed to stand at a lower temperature (25°); however, the time necessary to reach maximum absorbance was 144 hr.

RESULTS AND DISCUSSION

The red chromophore described exhibits a peak at 520 nm. The color is stable for 30 min. and then slowly fades on standing. An effort was made to quantitate the color reaction for cephalexin; however, a nonlinear relationship was observed in the concentration range from 0.25 to 1.0 mg./ml.

Effect of Reagent Concentrations on Color Formation—A series of test tubes, each containing 2.0 ml. of an aqueous solution of cephalexin (1.0 mg./ml.), was prepared. Varying volumes of reagents (±0.1 ml. of that specified in the *Recommended Procedure*) were added to the tubes and the test was completed. No significant difference in the extent of color formation was found in the series of solutions tested.

Effect of Heating Time and Temperature on Color Formation—A series of tubes, each containing 2.0 ml. of a solution of cephalexin (1.0 mg./ml.), was treated with the volume of reagents specified in the *Recommended Procedure* and heated in a boiling water bath for varying periods of time. The heating time that resulted in the greatest extent of color formation was 3 min. A similar series of tubes was heated at varying temperatures for 3 min.; 100° was the temperature that resulted in the greatest extent of color formation in the time specified.

Effect of Predegradation of Compound on Color Formation—Urine samples containing cephalexin were tested by this technique. It became apparent that the color formation was more intense if the solution stood at room temperature for several days prior to testing. Investigation of this phenomenon revealed that the pH of the solution was definitely related to the extent and rate of color formation observed.

The maximum rate and extent of color formation were achieved on testing a solution of cephalexin that had been buffered at pH 7 and allowed to stand at room temperature prior to the test. The time of standing at pH 7 (25°) required to obtain a maximum absorbance in the test was 6 days for cephalexin solutions and 2 days for cephaloglycin solutions. This increase in color formation was concurrent with the degradation of the cephalosporin prior to the test, as evidenced by the decrease in UV absorbance at the nucleus wavelength, approximately 260 nm.

Compounds Tested—Several compounds were tested by the recommended procedure to determine the specificity of the color reaction. The following compounds gave a positive (+) or negative (−) response to the test:

cephalexin	+
cephaloglycin	+
desacetyl cephaloglycin	+
cephaloglycin lactone	+
D-phenylglycine	−
7-aminocephalosporanic acid	−
7-aminodesacetoxycephalosporanic acid	−
sodium cephalothin	−
cephaloridine	−
sodium cephalosporin C	−
ampicillin (25–50 mg./ml.)	+

The sensitivity of the color reaction for cephalosporin derivatives was much greater than that observed for the corresponding penicillin analogs. The failure of the phenylalanyl derivative of 7-aminocephalosporanic acid and the indole derivative of 6-aminopenicillanic acid to respond was puzzling. The necessity of the presence of a heterocyclic or aromatic ring immediately adjacent to the α-amino group was conjectured. This theory could not be fully tested since an appropriate derivative was not found [$RCH(NH_2)CO$—ceph, where R is an aliphatic chain].

REFERENCES

(1) J. L. Spencer, E. H. Flynn, R. W. Roeske, F. Y. Siu, and R. R. Chauvette, *J. Med. Chem.*, **9**, 746(1966).

(2) C. W. Ryan, R. L. Simon, and E. M. Van Heyningen, *ibid.*, **12**, 310(1969).

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Influence of Chain Length on Oil–Water Ion-Pair Partitioning Behavior of *p*-Alkylpyridinium Chlorides

K. C. YEH and W. I. HIGUCHI▲

Abstract □ The intrinsic partition coefficients (k_i) of *p*-alkylpyridines and the extraction constants (K_e) of the corresponding ion-pairs of the protonated base chlorides were determined in chloroform–water and octanol–water systems. A linear relationship with unit slope was found between log (k_i) and log (K_e). This result has been explained on the basis that, because of methylene (CH_2) increments occurring distantly from the polar portion of the molecule, their influence upon the oil-water partition coefficient would be expected to be the same for the free base and the ion-pair.

Keyphrases □ *p*-Alkylpyridinium chlorides—influence of chain length on oil-water ion-pair partitioning behavior □ Ion-pair partitioning behavior, oil–water—influence of chain length using *p*-alkylpyridinium chlorides □ Chain length—influence on oil–water ion-pair partitioning behavior of *p*-alkylpyridinium chlorides □ Partition coefficients, *p*-alkylpyridinium chlorides—chloroform–water and octanol-water systems □ Extraction constants, *p*-alkylpyridinium chlorides—chloroform–water and octanol-water systems

There has been increased interest in the understanding of substituent effects upon the oil–water partitioning tendencies of drugs because of the possible correlation of the latter with drug absorption efficiency and biological availability in general. Studies on rat intestinal drug absorption (1, 2) and human buccal absorption

(3–5) showed that substituent effects upon the absorption rate can be related *via* physical transport models to the oil–water partition tendencies.

An observation was recently made (1, 2, 4, 5) that the relevant lipid biophase involved in drug absorption may be less nonpolar than previously believed; therefore, the partitioning and transport of ion-pairs (6–9) may be important in certain instances. The present article reports upon a comparative study of the influences of the CH_2-substitution on the partitioning tendencies of alkylpyridines and the corresponding protonated pyridine–chloride ion-pairs.

EXPERIMENTAL[1]

Materials—Straight-chain *p*-alkylpyridines were synthesized by the method of Wilbaut and Hey (10). Details of experimental procedures, such as the purification of each homolog, will be published elsewhere (11). The apparent pKa of each conjugate acid was determined spectrophotometrically using the method of Edwards (12).

Determination of Partition Coefficient—Aliquot amounts of the organic solvent (either 1-octanol or chloroform) were mixed with aliquot amounts of the aqueous phase at the desired pH. The aqueous layer contained 1 *M* sodium chloride as the swamping electrolyte together with 0.01 *M* each of the citrate and phosphate buffers. Partition coefficients were determined after 24 hr. of mild shaking in a water bath maintained at 25° at concentrations below the CMC of each conjugate acid. Concentrations in the aqueous layer were analyzed on a recording spectrophotometer[2]. Concentrations in the oil phase were calculated by a modified mass balance method (11).

Chloride Determinations—After determining the apparent partition coefficients in a chloroform–aqueous system, the oil layer that contained alkylpyridinium–chloride ion-pairs was mixed with a few drops of 5% aqueous sodium carbonate solution and evaporated to dryness at room temperature. Aliquots of water were added to the residues. The mixtures were washed three times with *n*-heptane, and the aqueous layers were then titrated for their chloride contents[3].

RESULTS AND DISCUSSION

Figure 1 shows the pH profile of the apparent partition coefficients of alkylpyridines in the chloroform–water system. Results of similar patterns have been observed in the octanol–water system. Based on this ion-pair model, the apparent partition coefficient k' (13) may be expressed as:

$$k' = \frac{[B]_o + [HBCl]_o}{[B]_w + [HB^+]_w} \qquad \text{(Eq. 1)}$$

where:

$[B]_o$ = concentration of free base in oil phase
$[B]_w$ = concentration of free base in water
$[HBCl]_o$ = concentration of ion-pair in oil phase
$[HB^+]_w$ = concentration of conjugate acid in water

If the oil phase does not contain ion-pairs, there will be a linear relationship between log (k') and pH when the pH is below the apparent pKa of the conjugate acid. The contribution of the ion-pair partitioned into the oil phase may be shown in the following expression:

$$f = \frac{K_e[H^+]_w[Cl^-]_w}{K_a k_t} \qquad \text{(Eq. 2}a\text{)}$$

[1] A Beckman model 1019 research pH meter was used for all pH measurements.
[2] Cary model 14.
[3] A model 4-2000 Standard Buchler-Cotlove Chloridometer was used.

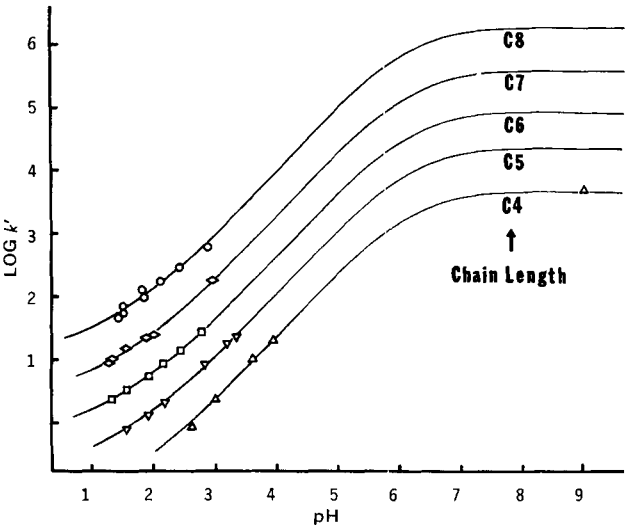

Figure 1—*Apparent partition coefficient–pH profile of alkylpyridines in chloroform–water system.*

or:

$$\log (f) = \log (K_e) - \log (k_t) + \log [Cl^-]_w + (pKa - pH) \qquad \text{(Eq. 2}b\text{)}$$

where:

f = $[HBCl]_o/[B]_o$, and is a measure of the relative concentration of the ion-pair in oil phase
K_e = extraction constant of the ion-pair
= $[HBCl]_o/[HB^+]_w[Cl^-]_w$
K_a = apparent dissociation constant of the conjugate acid (pKa 6.332 for all substituted alkylpyridines in 1 *M* sodium chloride)
k_t = intrinsic partition coefficient of free base
= $[B]_o/[B]_w$

The equation is self-explanatory, *i.e.*, the factor f is proportional to the concentration of the hydrogen ion and the monovalent chloride ion in the water phase. It is inversely proportional to the intrinsic partition coefficient of free base and to the apparent dissociation constant of the conjugate acid. Based on experimental data in the octanol–water system, the increasing dominance of the

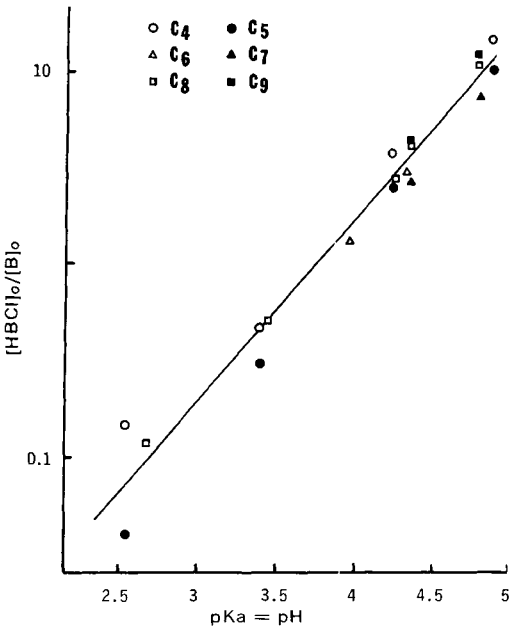

Figure 2—*Effect of pH on the contribution of ion-pairs in octanol phase.*

Table I—Estimated Partition Coefficients and Extraction Constants

Alkyl Chain Length	Octanol–Water log (k_t)	Octanol–Water log (K_e)	Chloroform–Water log (k_t)	Chloroform–Water log (K_e)
Butyl-	3.128	−0.671	3.737	−0.725[a]
Amyl-	3.750	−0.137	4.380	−0.524
Hexyl-	4.348	0.489	5.006	0.188
Heptyl-	5.008	1.074	5.653	0.744
Octyl-	5.542	1.782	6.331	1.448
Nonyl-	6.109	2.405	—	—

[a] There is indication that the error in estimating this value is very large.

ion-pair with decreasing pH is shown in Fig. 2. The concentration of ion-pairs in the organic phase has been quantitatively verified by the chloride assay.

Equation 1 may be further reduced to the following expression in acid media of 1 M sodium chloride solution, where $[Cl^-]_w$ = 1.0 and pH $\ll$ pKa:

$$\log (k_t) = \text{pKa} - \text{pH} + \log (k' - K_e) \qquad \text{(Eq. 3)}$$

The simultaneous estimation of k_t and K_e for each homolog compound was performed numerically on an IBM 360/67 digital computer. Results are shown in Table I. It is of interest to note that the octanol data yield a Hansch π value of 0.61 ± 0.01 for CH_2 group, which is somewhat larger than the 0.5 value reported by Hansch and Anderson (14). This difference might be due to the salting-out effect of the 1.0 M sodium chloride used in the present study. For both the octanol and chloroform systems, the π values for CH_2 group were found to be essentially constant over the entire range of the compounds seen in Table I. This suggests that the hydrophobic coiling (15) of the alkyl side chain in water, if it is important, does not seem to influence significantly the free energy of the water-to-oil transfer of the CH_2 groups.

Figure 3 shows plots of log (K_e) *versus* log (k_t). As can be seen, these gave straight lines with slopes of 1.036 and 0.995, respectively. These two values may be considered to be equal to 1.0 within the uncertainty of the data.

To understand these results, it must be realized that the extraction constant is actually equal to the product of two constants. The first one is the formation constant of the ion-pair in the aqueous phase (13). The other is the partition coefficient of the ion-pairs. Since the formation of the protonated pyridine–chloride ion-pair in water probably involves mainly the electrostatic interaction between the chloride ion and the polar portion of the alkylpyridinium ion, the stability constant of the ion-pair may not be expected to vary when distant methylene groups are added to the alkyl side chain. On the other hand, the partition coefficient of an alkyl derivative has been shown to increase exponentially with the chain length (14, 16). The linear relationship and the identical slopes observed in Fig. 3 suggest that this ion-pair system also follows this rule. This can be rationalized on the basis that the addition of a methylene group distant from the polar portion of the molecule would be expected to have the same effect upon the partition coefficient of the parent amine or that for the ion-pair.

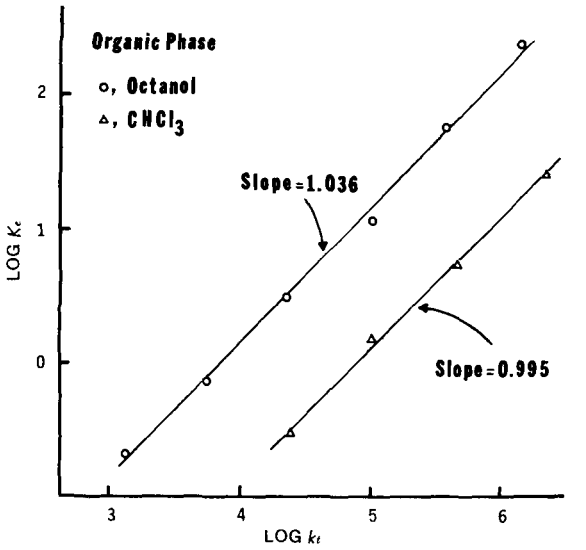

Figure 3—*Relationship between extraction constants and intrinsic partition coefficients.*

REFERENCES

(1) F. A. Wilson, V. L. Sallee, and J. M. Dietschy, *Science*, **174**, 1031(1971).

(2) N. F. H. Ho, J. T. Doluisio, and W. I. Higuchi, *J. Pharm. Sci.*, in press.

(3) A. H. Beckett and A. C. Moffat, *J. Pharm. Pharmacol., Suppl.*, **20**, 239S(1968).

(4) N. F. H. Ho and W. I. Higuchi, *J. Pharm. Sci.*, **60**, 537 (1971).

(5) K. R. M. Vora, W. I. Higuchi, and N. F. H. Ho, paper presented to APhA Academy of Pharmaceutical Sciences, Houston meeting, Apr. 1972.

(6) K. S. Murthy and G. Zografi, *J. Pharm. Sci.*, **59**, 1281(1970).

(7) R. L. Hull and J. A. Biles, *ibid.*, **53**, 869(1964).

(8) T. Higuchi and K. Kato, *ibid.*, **55**, 1080(1966).

(9) R. Modin and G. Schill, *Acta Pharm. Suecica*, **7**, 585(1970).

(10) J. P. Wilbaut and J. W. Hey, *Rec. Trav. Chim.*, **72**, 513 (1953).

(11) K. C. Yeh and W. I. Higuchi, *J. Pharm. Sci.*, in press. (See also K. C. Yeh, Ph.D. thesis, University of Michigan, Ann Arbor, Mich., 1972.)

(12) L. J. Edwards, *Trans. Faraday Soc.*, **46**, 723(1950).

(13) T. D. Doyle and J. Levine, *Anal. Chem.*, **39**, 1282(1967).

(14) C. Hansch and S. M. Anderson, *J. Org. Chem.*, **32**, 2583 (1967).

(15) G. Nemethy and H. A. Scheraga, *J. Chem. Phys.*, **36**, 3382, 3401(1962).

(16) L. Alders, *Appl. Sci. Res.*, **A4**, 171(1954).

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