Partition Coefficients of Biologically Important Pyrimidines and Purines [1]

Cyril Párkányi* and Dah-Chung Shieh [2]

Department of Chemistry, The University of Texas at El Paso, El Paso, Texas 79968-0513, U.S.A.

Z. Naturforsch. 41b, 1580–1582 (1986); received January 10, 1986

Pyrimidines, Purines, Partition Coefficients, Extraction, Solubility

Partition coefficients of sixteen biologically important pyrimidines and purines between an organic phase and water have been determined and the best organic solvents for their extraction have been suggested.

Distribution (partition) of an organic substance between two liquid phases, usually water and an organic solvent, plays an important role in extraction, partition chromatography, and various other separation processes used in many areas of chemistry.

Partition (distribution) coefficient, P, which is the quantity used to characterize the distribution of a solute between two immiscible solvents (one of which is normally water), is defined as $P = C_o / C_w$, where $C_o$ is concentration of the extractable species in the organic solvent and $C_w$ is concentration of the extractable species in the aqueous layer [3]. In most cases, partitioning can be treated by methods of classical thermodynamics. Partition coefficients represent a useful criterion for the selection of a suitable solvent and the choice of optimum conditions for extraction. Also, distribution of biomolecules between water and an organic phase often parallels their biological activity.

As a continuation of our systematic studies of ground- and excited-state physical properties of biologically important pyrimidines and purines [4, 5], we have decided to investigate the solubility of sixteen pyrimidines and purines in several organic solvents and to determine the corresponding partition coefficients. The pyrimidines and purines included in our study were nucleic acid components (uracil, thymine, cytosine, adenine, guanine), alkaloids (hypoxanthine, theobromine, theophylline, caffeine), and cancerstatic drugs or otherwise biologically active compounds (5-fluoro-, 5-bromo-, 6-mercaptopurine) (Scheme 1).

In the past twenty years, the biological activity of pyrimidines and purines has been widely studied. Because partitioning between the aqueous phase and an organic phase can serve as a model for how a biomolecule passes through the membranes in a living organism, partition coefficients of these compounds are of interest. Partition coefficients are also important in the use of various solvents to extract caffeine from coffee and other food products to obtain their decaffeinated counterparts. In addition to the partition coefficients of caffeine [6–9], the other purines studied were theobromine and theophylline [10–13]. Several additional important studies in this

* Reprint requests to Prof. Dr. Cyril Párkányi.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0340–5087/86/1200–1580 $01.00/0

scheme 1.
area have appeared in the literature [14—16]. However, no data are available in the literature for most of the compounds for which they are reported in our study.

Solubility of heterocyclic compounds was discussed in detail by Pfleiderer [17] and by Albert [18]. The lone-electron pairs on doubly-bonded (pyridine-like) nitrogen atoms can readily form hydrogen bonds with water and thus make such \( \pi \)-azaheterocycles more soluble in water than the corresponding hydrocarbons or azaheterocycles in which the lone-electron pair on the nitrogen atom is a part of the aromatic six \( \pi \)-electron system (pyrrole). Thus, pyridine is completely miscible with water whereas the solubility of pyrrole is relatively poor. Also, substituents with bondable hydrogen often cause a significant decrease in water solubility of heterocycles with pyridine-like nitrogen atoms due to intramolecular or intermolecular hydrogen bonding.

The results obtained in our study are summarized in Table I and can be used to determine which of the solvents is the best one for extraction of a particular compound from an aqueous phase. Ethyl acetate is the most practical solvent in most cases, closely followed by diethyl ether, with chloroform and di-\( n \)-butyl ether as additional useful solvents. In addition to the solvents mentioned above, the other solvents used in this work were benzene, carbon tetrachloride, and \( n \)-octane.

In the pyrimidine series, uracil is somewhat less soluble in ethyl acetate than thymine due to the presence of a hydrophobic methyl group in thymine which increases its solubility in organic solvents. However, as expected, the introduction of an amino group in the case of cytosine decreases solubility in organic solvents (possible hydrogen bonding). In the series of halosubstituted uracils, their solubility in ethyl acetate increases in the sequence 5-fluorouracil < 5-bromouracil < 5-iodouracil, i.e., with decreasing electronegativity of the halogen. The higher solubility of halouracils in organic solvents is in agreement with Pfleiderer’s suggestion that the lower solubility of halosubstituted heterocycles in water is due to the hydrophobic character of the halogens [17].

In the purine series, the introduction of highly polar, hydrogen-bond forming groups such as an amino or an oxo group decreases the solubility in organic solvents: the partition coefficients of adenine and guanine are only 10% of the corresponding value for unsubstituted purine. The partition coefficient of hypoxanthine is only 5% of that of purine. In the series of oxo derivatives, the solubility in ethyl acetate increases in the sequence hypoxanthine < theobromine (one methyl group in the pyrimidine ring) < theophylline (two methyl groups in the pyrimidine ring) < caffeine (totally methylated). 6-Chloropurine and 6-mercaptopurine which do not possess any oxo or amino groups are relatively well soluble in organic solvents. Pfleiderer suggested that the lower solubility of heterocyclic mercapto derivatives in water is due to the formation of intermolecular hydrogen bonds between two molecules [17]. In many cases (most of which are not shown in Table I), the partition coefficients for the various pyrimidines and purines are extremely low reflecting very poor solubility of these polar compounds in organic solvents. In general, more polar solvents work better than nonpolar solvents.

The results obtained in this work should prove useful for selection of a suitable solvent for extraction of pyrimidines and purines from aqueous solutions or from the various materials in which they occur.

---

### Table I. Partition coefficients, \( P \), of selected pyrimidines and purines.a

| No. | Compound | AcOEt | Et\(_2\)O | Third solvent
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Uracil</td>
<td>0.14</td>
<td>0.07</td>
<td>0.09 (O)</td>
</tr>
<tr>
<td>2</td>
<td>Thymine</td>
<td>0.18</td>
<td>0.09</td>
<td>0.05 (BE)</td>
</tr>
<tr>
<td>3</td>
<td>Cytosine</td>
<td>0.11</td>
<td>0.11</td>
<td>0.06 (C)</td>
</tr>
<tr>
<td>4</td>
<td>5-Fluorouracil</td>
<td>0.25</td>
<td>0.10</td>
<td>0.04 (C)</td>
</tr>
<tr>
<td>5</td>
<td>5-Bromouracil</td>
<td>0.66</td>
<td>0.14</td>
<td>0.05 (C)</td>
</tr>
<tr>
<td>6</td>
<td>5-Iodouracil</td>
<td>1.04</td>
<td>0.19</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>Purine</td>
<td>0.21</td>
<td>0.08</td>
<td>0.08 (O)</td>
</tr>
<tr>
<td>8</td>
<td>Adenine</td>
<td>0.02</td>
<td>0.06</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>Guanine</td>
<td>0.02</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>6-Methylpurine</td>
<td>0.11</td>
<td>0.07</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>6-Chloropurine</td>
<td>1.03</td>
<td>0.25</td>
<td>0.08 (C)</td>
</tr>
<tr>
<td>12</td>
<td>6-Mercaptopurine</td>
<td>0.49</td>
<td>0.43</td>
<td>1.34 (BE)</td>
</tr>
<tr>
<td>13</td>
<td>Hypoxanthine</td>
<td>0.10</td>
<td>0.05</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>Theobromine</td>
<td>0.23</td>
<td>0.07</td>
<td>0.49 (C)</td>
</tr>
<tr>
<td>15</td>
<td>Theophylline</td>
<td>0.34</td>
<td>0.15</td>
<td>0.30 (C)</td>
</tr>
<tr>
<td>16</td>
<td>Caffeine</td>
<td>0.80</td>
<td>0.19</td>
<td>4.70 (C)</td>
</tr>
</tbody>
</table>

---

*a The values were obtained with the original concentration of the aqueous solution \( 1 \times 10^{-4} \) M in the substrate; b B = benzene, BE = di-\( n \)-butyl ether, C = chloroform, O = \( n \)-octane; c in benzene, \( P = 0.05 \); d insoluble; e in \( n \)-octane, \( P = 0.06 \); f in \( n \)-octane and in benzene, \( P = 0.05 \); g in chloroform, lit. [8] gives \( P = 0.4 \); h in chloroform, lit. [8] gives \( P = 0.3 \); i in chloroform, from ref. [13]. Additional solvents: carbon tetrachloride, \( P = 0.20 \) (lit. [13] gives 0.12); \( n \)-octane, \( P = 0.03 \) (lit. [13] gives 0.03).
Experimental

Materials

Cytosine, 5-fluorouracil, adenine, 6-chloropurine, 6-mercaptopurine, hypoxanthine (6-hydroxypurine), and theophylline were purchased from the United States Biochemical Corporation, Cleveland, Ohio. 5-Iodouracil, purine, guanine, and 6-methylpurine were obtained from Chemical Dynamics Corporation, South Plainfield, New Jersey. Theobromine was the product of Eastman Kodak Company, Rochester, New York, and uracil, thymine, 5-bromouracil, and caffeine were from Aldrich Chemical Company, Milwaukee, Wisconsin.

Solvents

The following seven solvents were used in this work: n-octane, benzene, carbon tetrachloride, chloroform, di-n-butyl ether, diethyl ether, and ethyl acetate. The solvents were reagent grade.

Instruments

Electronic absorption spectra were measured on a Cary 118 spectrophotometer. A Burrel shaker was used to mix water and organic solvents.

Analytical procedure

Aqueous solutions of pyrimidines and purines used in the study were $1 \times 10^{-5}$, $5 \times 10^{-5}$, and $1 \times 10^{-4}$ M in the substrate. An identical volume (10 ml) of each of these three solutions was used. An equal volume (10 ml) of the appropriate organic solvent was added and the mixture was shaken for 12 h in a shaker at 25 °C. Then it was left for 12 h so that an equilibrium between the two phases could be established. In most cases, only small differences (less than 10%) were observed for partition coefficients obtained at different concentrations. The estimated error for the individual determinations is ±10%. However, this is not true for those cases where the solubility was extremely low.

The concentrations of pyrimidines and purines used in this study were determined spectrophotometrically. The concentrations of the compounds in organic solvents were obtained from the difference between the concentration of the original aqueous solution before extraction, and the concentration determined spectrophotometrically in the aqueous solution after equilibration. The following wavelengths were used for the spectrophotometric determinations (the wavelength in nm is shown in parentheses): uracil (259), thymine (264), cytosine (268), 5-fluorouracil (266), 5-bromouracil (274), 5-iodouracil (284), purine (262), adenine (269), guanine (274), 6-methylpurine (269), 6-chloropurine (264), 6-mercaptopurine (324), hypoxanthine (264), theobromine (274), theophylline (269), and caffeine (274).

Financial support of this work by the Welch Foundation, Houston, Texas (Grant AH-461) is gratefully acknowledged.

[1] This work was presented in part at the Combined 38th Southwest-6th Rocky Mountain Regional Meeting of the American Chemical Society, El Paso, Texas, December 1–3, 1982.
[2] Present address: Department of Chemistry, Ohio State University, 140 West 18th Avenue, Columbus, Ohio 43210.