The Solvent Extration of Amino Acids with Crown Ether[1)]

Hideyuki Noguchi,* Hiroyoshi Nakamura, Masatoshi Nagamatsu, and Masaki Yoshio

Faculty of Science and Engineering, Saga University, Saga 840

(Received July 3, 1981)

Protonated amino acids were extracted with crown ether and Metanil Yellow anion into 1,2-dichloroethane. The overall extraction constants (K_{ex}) for the 1:1:1 complex, AHLIn, of 18-crown-6 (L) with protonated amino acid (AH^+), and the Metanil Yellow anion (In^-) between 1,2-dichloroethane and water have been determined at 25.0 °C. The values of the extraction constants (log K_{ex}) were found to be 5.2, 6.2, 6.4, 6.3, 5.5, 6.2, and 5.5 for L-α-alanine, L-valine, L-leucine, L-phenylalanine, β-alanine, L-lysine, and aspartic acid respectively at 25.0 °C. The dependence of the varieties of crown ether on the extractability of L-leucine has been investigated. The highest extractability was obtained when water-soluble crown ether was used.

The solvent extraction of cationic metal ions with crown ether (L) has been widely carried out,[2)] the results indicate the dependence of the extractability on the cavity size of crown ether. Very few studies of the solvent extraction of organic species with crown ether have, however, been done. This work has been carried out in order to study the extraction of some organic species, amino acids, with crown ether. It is well known that the ammonium ion and protonated amine make a relatively stable complex with crown ether. A small difference in stability constant between primary and secondary amine complexes with crown ether has been observed.[3)] The present authors have previously indicated that the selectivity of crown ether for various type of amines can be improved by using the solvent-extraction method.[4)] Most amino acids include the $-NH_2$ group, its protonated form being able to complex with crown ether.

Therefore, the solvent extraction of amino acids (A) with crown ether as Metanil Yellow salt (AH^+LIn^-) has been attempted in this work. Metanil Yellow (In^-) was selected as the counter anion because of its larger dissociation constant and because it has its absorption in the visible region for spectrophotometric analysis.

Experimental

Reagents. 18-Crown-6 was synthesized by Gokel's method[5)] and recrystallized twice from acetonitrile. The acetonitrile was removed under a high vacuum (0.5 mmHg†) over a 5-h period. Crown ether shows no significant hydroxyl or cyano vibration in the 3500 and 2000 cm^{-1} infrared region. "Dibenzo-18-crown-6" was synthesized by the method of Pedersen[6)] and recrystallized from benzene. The polyether thus obtained showed a melting point and an infrared spectrum identical with those of an authentic sample. "Dicyclohexyl-18-crown-6" and the other crown ethers were obtained from the Nisso Co. and were used without further purification. The 1,2-dichloroethane and other reagents were of an analytical or special grade, and were used without purification.

Extraction Procedures. A typical extraction procedure was as follows: 25 ml of an aqueous solution containing 4×10^{-4} M (1 M=1 mol/dm^3) L-leucine and 6×10^{-5}—2.4×10^{-4} M Metanil Yellow was shaken with 10 ml of 1,2-dichloroethane containing 2×10^{-3}—1.6×10^{-2} M crown ether when 18-crown-6 was used. After equilibrium had been reached, the absorbance in the organic phase was measured at 404 nm with a Hitachi 124 spectrophotometer in a 10 mm cell.

HIn, HLIn, and AHLIn contribute to the absorbance in the organic phase at 404 nm. HIn, however, did not show the absorbance in the pH range from 2 to 4. Therefore, the absorbance of AHLIn can be corrected by the blank test, which is related to that of HLIn. The constant pH value of 2.8±0.1 was selected in order to produce a precipitate at the phase boundary in the pH range lower than 2.

† 1 mmHg≈133.322 Pa.

Results and Discussion

When a protonated amino acid (AH^+) forms a complex with a crown ether, the complex can be extracted as an ion-pair with a large anion (In^-); the distribution ratio of amino acid, D, in the pH range 2—4 can be written as:

$$D = \frac{[AHLIn]_{org}}{[A]+[AH^+]}. \quad (1)$$

Here, K_{ex} is the overall extraction constant for the complex:

$$K_{ex} = \frac{[AHLIn]_{org}}{[AH^+][L]_{org}[In^-]}. \quad (2)$$

Combining these two equations gives the following equation:

$$D = K_{ex}[L]_{org}[In^-]\left(1+\frac{K}{[H^+]}\right)^{-1}, \quad (3)$$

where K is the dissociation constant of protonated amino acid.

Under the condition of a constant pH, the distribution ratio as a function of the concentration of the crown ether in the organic phase at 25.0 °C showed a straight line with a slope of 1, while that as a function of the Metanil Yellow also showed a straight line with a slope of 1, as is shown in Figs. 1 and 2. These results show that the 1,2-dichloroethane extracts is a 1:1:1 complex composed of crown ether, amino acid, and Metanil Yellow. From Figs. 1 and 2, the values of log K_{ex} are found to be 5.2, 6.2, 6.4, 6.3, 5.5, 6.2, and 5.5 for L-α-alanine, L-valine, L-leucine, L-phenylalanine, β-alanine, L-lysine, and L-aspartic acid respectively at 25.0 °C. As was seen in the previous paper,[4)] the value of K_{ex} depends greatly on the number of hydrogen atoms available for hydrogen bonding with crown ether. In this case, the difference in K_{ex} is very small, because all of the amino acids investigated have the same number

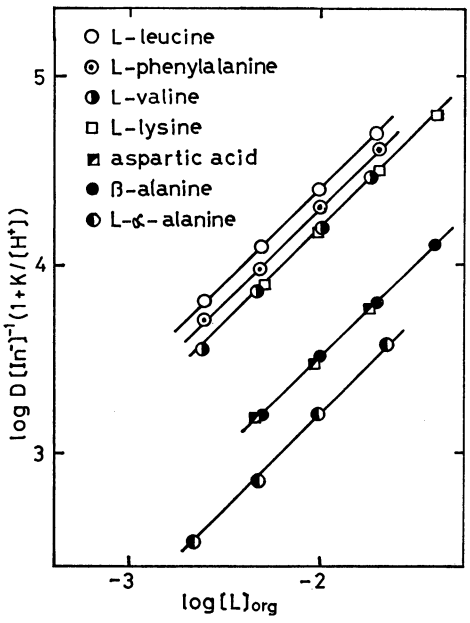

Fig. 1. Plots of $\log D\,[In^-]^{-1}\,(1+K/[H^+])$ *vs.* $\log [L]_{org}$ for amino acids at 25.0 °C.

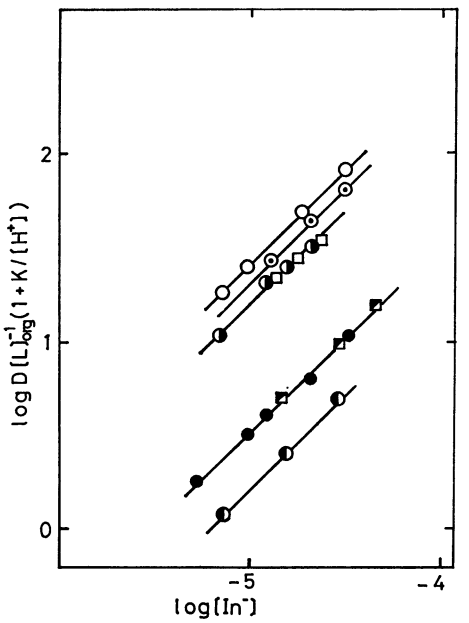

Fig. 2. Plots of $\log D\,[L]_{org}^{-1}\,(1+K/[H^+])$ *vs.* $\log [In^-]$ for amino acids at 25.0 °C.

of hydrogen atoms, $-NH_3^+$. The small difference in K_{ex} may be due to the effect of the size of the amino acids, which was also shown in the previous paper.[4]

The dependence of the varieties of crown ether on the extractability of amino acid, L-leucine, was also investigated. The extractability of L-leucine decreased in the following order: 18-crown-6>"dicyclohexyl-18-crown-6">"dibenzo-18-crown-6".

18-Crown-6, which is very soluble in water, gives rise to the most enhanced extractability, indicating that extractability is increased with an increase in the solubility of crown ether in water when the ring size of crown ether is the same.

Table 1. Extraction constants and related values of L-leucine at 25 °C

	$\log K_{ex}$	$K_{d,L}$	K_{dAHLIn}/K_{AHLIn}[a]
18-Crown-6	6.4	1.1	2.8×10^6
"Dicyclohexyl-18-crown-6"	6.1	760	9.6×10^8
"Dibenzo-18-crown-6"	4.5	3200	1.0×10^8

a) Values calculated from K_{ex} and $K_{d,L}$ using Eq. 4.

The overall extraction constant could be written as follows:

$$K_{ex} = \frac{K_{d,AHLIn}K_{AHLIn}}{K_{d,L}}, \quad (4)$$

where $K_{d,L}=[L]_{org}/[L]$, $K_{d,AHL\,In}=[AHL\,In]_{org}/[AHL\text{-}In]$, and $K_{AHL\,In}=[AHLIn]/[AH^+][L][In^-]$. The overall extraction constants (25.0 °C) obtained are shown in Table 1.

Table 1 also shows the values of $K_{d,L}$ and $K_{d,AHL\,In}$ $K_{AHL\,In}$, which were calculated from the values of K_{ex} and $K_{d,L}$.

Equation 4 shows that the trend for K_{ex} reflects $K_{AHL\,In}$ and the distribution coefficients of the AHLIn and L species. It is well known that the hydrophobic species are extracted more easily than the hydrophilic species. The values of $K_{d,AHL\,In}$ $K_{AHL\,In}$ increase with an increase in the hydrophobic nature of crown ether, which may reflect the extractability of the compound, AHLIn. However, the effect of $K_{d,L}$ on the extractability is much greater than that of the last term in Table 1.

The extraction of L-leucine with crown ether having a bigger pore size than 18-crown-6 was also investigated. The same trend of extractability was obtained: "dicyclohexyl-24-crown-8">"dibenzo-24-crown-8". The extractability of L-leucine with the 18-membered crown ethers is much greater than with the 24-membered.

Thus, the distribution coefficient of the crown ether, $K_{d,L}$ in Eq. 4 is the most important factor in determining the magnitude of K_{ex} for amino acid. Thus, a soluble crown ether in water, such as 18-crown-6, may be considered a promising reagent for the separation and extraction of amino acids.

References

1) Application of Crown Ether to Chemical Analysis. IV.

2) T. Sekine, H. Wakabayashi, and Y. Hasegawa, *Bull. Chem. Soc. Jpn.*, **51**, 645 (1978); T. Sekine, K. Shioda, and Y. Hasegawa, *J. Inorg. Nucl. Chem.*, **41**, 571 (1979); H. Sumiyoshi, K. Nakahara, and K. Ueno, *Talanta*, **24**, 763 (1977); T. Iwachido, M. Kimura, and K. Tōei, *Chem. Lett.*, **1976** 1101; A. Sadakane, T. Iwachido, and K. Tōei, *Bull. Chem. Soc. Jpn.*, **48**, 60 (1975); Y. Takeda and H. Katō, *ibid.*, **52**, 1027 (1979); Y. Takeda and H. Gotō, *ibid.*, **52**, 1920 (1979); Y. Takeda, *ibid.*, **52**, 2501 (1979); P. R. Danesi, H. Meider-Gorican, R. Chiarizia, and G. Scibona, *J. Inorg. Nucl. Chem.*, **37**, 1479 (1975); Y. Marcus and L. E. Aser,

J. Phys. Chem., **82**, 1246 (1978).

3) J. L. Roberts, R. E. McClintock, Y. El-Omrani, and J. W. Larson, *J. Chem. Eng. Data*, **24**, 79 (1979); R. M. Izatt, N. E. Izatt, B. E. Rossiter, J. J. Christensen, and B. E. Haymores, *Science*, **199**, 995 (1978); H. K. Frensdorff, *J. Am. Chem. Soc.*, **93**, 600 (1971); R. M. Izatt, D. P. Nelson, J. H. Rytting, and J. J. Christensen, *ibid.*, **93**, 1619 (1971).

4) H. Noguchi, M. Yoshio, K. Kawahara, H. Nakamura, and M. Nagamatsu, *Anal. Lett.*, **13A**, 271 (1980).

5) G. W. Gokel and D. J. Cram, *J. Org. Chem.*, **39**, 2445 (1974).

6) C. J. Pedersen, *J. Am. Chem. Soc.*, **89**, 7017 (1967).