THE SOLUBILITIES OF THE l-DIHALOGENATED TYROSINES IN ETHANOL-WATER MIXTURES AND CERTAIN RELATED DATA*

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The solubility relationships of the amino acids in ethanol-water mixtures are important both from the theoretical as well as from the practical standpoint. Decrease in the dielectric constant of the solvent, such as is produced by the addition of ethanol or acetone, is usually accompanied by a decrease in the solubilities of the amino acids (1). l-Tyrosine behaves in this respect like the majority of the aliphatic amino acids. However, in the case of the dihalogenated tyrosine compounds, it is found that their solubilities are markedly increased by the addition of ethanol to a certain concentration. Further addition of ethanol leads to a decrease in solubility. Evidently, factors other than the dielectric constant are concerned.

The importance of this fact is brought out in the preparation of diiodo-tyrosine, according to the procedure of Oswald (2). He used ethanol for purposes of crystallization. Excess iodine is thus removed. Very poor yields of diiodo-tyrosine are obtained. By following this procedure, it was found that the mother liquor, on cooling, would often become a transparent solid gel. This suggested that solvation, which usually increases solubility (3), had taken place. These observations led to the present investigation on the solubilities of l-tyrosine and its dihalogenated substitution products in ethanol-water mixtures.

The compounds used in the present investigation were prepared according to the procedures which have already been described (4). dl-Thyroxine was a Hoffmann-La Roche product. It was used without further purification. The technique employed in the determina-

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773
tion of solubility and the quantitative estimation of the amino acids was the same as that which has been given elsewhere (4). The concentrations of d/-thyroxine in the saturated solutions were obtained by iodine determinations. The amounts of ethanol in the ethanol-water mixtures were found by density determinations. All solubility determinations in alcohol-water mixtures were carried out at 25°. Due to the viscosity of certain of the solvents used for the solubility estimations of d/-thyroxine, the solubility of this compound was determined at 30°.

Experimental Results

Twenty solubility determinations on l-tyrosine in 5 different ethanol-water mixtures were carried out. The data are represented graphically by Curve I in Fig. 1 in which the logarithm of the solubility ratio, \( \frac{N_2}{N_2^o} \), is plotted against the mol fraction, \( N_s \), of ethanol in the solvent. The ratio, \( \frac{N_2}{N_2^o} \), refers the solubility of the amino acid in the ethanol-water mixture to its solubility in water. The solubility of l-tyrosine decreases as the concentration of ethanol is increased. Its solubility in ethanol \( (N_2 \times 10^8 = 0.5) \) is somewhat lower, but of the same order of magnitude as was found by Cohn and his coworkers (1) for a number of aliphatic amino acids. The ratio, \( \frac{N_2}{N_2^o} \), for tyrosine \( (1.0 \times 10^{-1}) \) is larger than for the other amino acids which have been reported due to the low solubility of l-tyrosine in water.

Dichloro-l-tyrosine, when recrystallized from water, contains water of crystallization (4). Thirty-two solubility determinations of dichloro-l-tyrosine (hydrated) in eight concentrations of ethanol-water mixtures were carried out. The results are represented graphically by Curve III in Fig. 1. The solubility of this compound increases as the mol fraction of ethanol in the mixture is increased until a maximum is reached. At this point the mol fraction of dichloro-l-tyrosine in the ethanol-water mixture is about four times larger than the mol fraction in its saturated aqueous solution. On further increase of ethanol, the solubility of dichloro-l-tyrosine is decreased.

In determining the solubility of dichloro-l-tyrosine (hydrated) in
ethanol, it was found that the solubility, after shaking the mixture for 3 days, was greater than was to be expected in relation to the solubility found in other ethanol-water mixtures. The solubility of this compound in ethanol with time is given in Table I. The excess of solute was found to have a different appearance after being in contact with

**Fig. 1.** The relationship between the logarithm of the solubility ratio and the mol fraction of ethanol in the alcohol-water mixture for L-tyrosine (Curve I), dibromo-L-tyrosine (Curve II), dichloro-L-tyrosine (Curve III), and diiodo-L-tyrosine (Curve IV). Maximum error in data: L-tyrosine and diiodo-L-tyrosine ± 5 per cent; dibromo- and dichloro-L-tyrosine ± 1.6 per cent.

ethanol for 6 days than when it was in contact with ethanol for 3 days. Microscopically it had a different crystal form (Fig. 2) than when crystallized from water (4). Recrystallization from hot ethanol gave the same crystal form as shown in Fig. 2. Analysis of this form showed it to be free of water of crystallization. The high solubility found after shaking for several days was probably due
to the presence of both forms of dichloro-\(l\)-tyrosine in the mixture. The determined solubility during this time represented the solubilities of the two forms. After all of the hydrated form had been changed to the anhydrous form, the solubility became constant and represents the solubility of the latter form in ethanol (plus the small amount of water resulting from that which represented water of crystallization).

Dibromo-\(l\)-tyrosine crystallizes from water in two forms depending on the conditions under which crystallization is carried out (4). Thirty-two solubility measurements of dibromo-\(l\)-tyrosine (anhydrous) in eight concentrations of ethanol-water mixtures were carried out. The results are represented graphically by Curve II in Fig. 1. As in the case with dichloro-\(l\)-tyrosine, the solubility increases as the mol fraction of ethanol is increased until a maximum is reached. This occurred at approximately the same concentration of ethanol as with dichloro-\(l\)-tyrosine. The mol fraction of dibromo-\(l\)-tyrosine at its maximum solubility is about four times as great as that in a saturated aqueous solution. Further addition of ethanol to the mixture led to a decrease in solubility. The solubility of dibromo-\(l\)-tyrosine in ethanol is approximately the same as in water.

Thirty-two solubility determinations of diiodo-\(l\)-tyrosine in eight concentrations of ethanol-water mixtures were carried out. The results are represented graphically by Curve IV in Fig. 1. As with the two other dihalogenated tyrosines, the solubility of this compound increases as the mol fraction of ethanol in the mixture is increased until

### TABLE I

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>Solubility (gm. per 1000 gm. solvent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3.768</td>
</tr>
<tr>
<td>8</td>
<td>4.131</td>
</tr>
<tr>
<td>24</td>
<td>4.015</td>
</tr>
<tr>
<td>72</td>
<td>3.950</td>
</tr>
<tr>
<td>120</td>
<td>1.914</td>
</tr>
<tr>
<td>144</td>
<td>1.889</td>
</tr>
<tr>
<td>168</td>
<td>1.906</td>
</tr>
</tbody>
</table>
a maximum is reached. The maximum occurs at a higher concentration of ethanol than in the case of the other dihalogenated tyrosines. At the maximum point the mol fraction of diiodo-l-tyrosine is about fifteen times as great as its solubility in water. The solubility in ethanol is considerably higher than in water. It was noted that the excess of solute in the higher ethanol-water mixtures changed in appearance from a crystalline substance to a transparent gel. It
resembled the material which is sometimes obtained when diiodo-\(l\)-tyrosine is recrystallized from ethanol.

The effect of temperature on the solubility of diiodo-\(l\)-tyrosine in an ethanol-water mixture (\(N_3 = 0.470\)) was also determined. At this concentration of ethanol, the solubility of diiodo-\(l\)-tyrosine is approximately at its maximum. Twelve solubility determinations were carried out at five different temperatures between 0° and 47.5°. The equation which expresses the solubility of diiodo-\(l\)-tyrosine as a function of temperature was calculated to be

\[
\ln N_3 = 4.01 \times 10^{-2} T - 19.808.
\]

On the assumption that the equation which was used to calculate the apparent differential heat of solution in water (4) can be applied in this case also, the value for \(\Delta H_{298^\circ} = 7090\) calories per mol at saturation. This value is somewhat less but of the same order of magnitude as that found by Dalton and Schmidt (5) in the case of a saturated aqueous solution of diiodo-\(l\)-tyrosine (7830 calories).

Attempts were made to determine the solubility of diiodo-\(l\)-tyrosine in propylene glycol. The amino acid is unstable in this solvent. The solution begins to turn brown within 24 hours. The solubility values at this time were twenty to twenty-five times larger than the solubility value of diiodo-\(l\)-tyrosine in water.

A few solubility determinations were carried out on \(dl\)-thyroxine. On the basis of iodine estimations, no measurable solubility in water was found. At 25°, the solubility in an ethanol-water mixture con-
taining 0.470 mol fraction of ethanol was 0.015 gm. per 1000 gm. of solvent. The solubility values of dl-thyroxine in various other solvents are given in Table II. Of those which were tried, propylene glycol was found to be the best solvent.

**DISCUSSION**

Cohn and his coworkers (1) have summarized the factors which affect the solubility of aliphatic amino acids in ethanol-water mixtures. When the volume fraction of ethanol is small, the logarithm of the solubility depends chiefly on the dielectric constant. The solubility relations of the longer hydrocarbon chain amino acids are more complex especially when the system contains large volume fractions of ethanol. In a general way, the solubility relations of L-tyrosine resemble those of the long hydrocarbon aliphatic amino acids.

The introduction of halogens into the hydroxyphenyl ring affects the solubility of the resulting compounds in ethanol-water mixtures to such a degree that the factors other than the dielectric constant appear to play important roles. The present data are not sufficient to warrant making precise conclusions. However, the nature of the solvent as well as the specific chemical groups in the molecule are factors which affect solubility. In the case of the dihalogenated tyrosine compounds at least two important factors operate to affect their solubility. One is the hydrocarbon chain. The effect of the addition of ethanol is to decrease its solubility. The presence of the hydroxyphenyl ring in the molecule is without decided influence. The effect of the presence of the halogens in the ring, probably as a result of orientation to the ethanol molecules, is to increase the solubility of the dihalogenated tyrosine compounds. This effect is opposite to that of the hydrocarbon chain. The halogen effect predominates over the effect of the latter up to a certain concentration of alcohol. With further increase in ethanol concentration, the effect of the hydrocarbon chain becomes more pronounced, with the result that the solubility decreases. The effect of the halogens continues to be considerable, however, with the result that the solubility of the dihalogenated tyrosine compounds in alcohol is decidedly greater than that of tyrosine.
SOLUBILITIES OF DIHALOGENATED TYROSINES

SUMMARY

1. The solubilities of l-tyrosine, dichloro-l-tyrosine (hydrated), dibromo-l-tyrosine (anhydrous), and diiodo-l-tyrosine in ethanol-water mixtures at 25° have been determined.

2. It was found that the solubilities of the dihalogenated substitution products of l-tyrosine are increased by addition of ethanol up to a certain concentration. Further addition of ethanol leads to a decrease in solubility. The solubility of l-tyrosine is decreased by addition of ethanol.

3. Dichloro-l-tyrosine (hydrated) was found to change to the anhydrous form when allowed to stand in the presence of ethanol.

4. The apparent heat of solution of diiodo-l-tyrosine in an ethanol-water mixture has been determined.

5. The solubility of dl-thyroxine at 30° has been determined in urea solution, ethanol, dioxan, ethylene glycol, and propylene glycol.

REFERENCES