THE COMBINATION OF CERTAIN FATTY ACIDS WITH LYSINE, ARGININE, AND SALMINE*

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(Received for publication, March 25, 1935)

The present work deals with the combinations which take place between certain fatty acids and arginine, lysine, and salmine. These compounds differ from those lipoproteins which have been synthesized by means of the Schotten-Baumann reaction (1). Under suitable conditions, fatty acids will combine with any base which is sufficiently strong to impart a low value to the hydrolysis constant of the resultant soap. The low solubility of the higher fatty acids in water makes it difficult to study the formation of such soaps by titrimetric methods. Ethanol-water mixtures may, however, be used for solvent purposes. The dissociation constants of a number of amino acids in ethanol-water mixtures have been reported (2). In the present communication, similar data are reported for some of the fatty acids. The titration curves of lysine, arginine, and salmine with certain fatty acids have been measured in alcoholic solution and the soaps have been prepared.

The amino acids used in this investigation were compounds of known purity. Acetic, propionic, and valeric acids were tested for purity by titration with sodium hydroxide in aqueous solution. Caprylic, capric, lauric, myristic, palmitic, stearic, oleic, and erucic acids were highly purified samples which were furnished generously by Dr. S. Lepkovsky. Salmine was prepared from a specimen of salmine sulfate which was prepared by the late Professor A. Kos-

* Aided by a grant from The Chemical Foundation, Inc., and the Research Board of the University of California.
We are indebted to the Cyrus M. Warren Fund of the American Academy of Arts and Sciences for the loan of the type K potentiometer.
† National Research Council Fellow in Medicine, 1933–34.
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sel, and presented by Professor S. Edlbacher. The titrimetric methods used have been previously described (2).

EXPERIMENTAL

Determination of $pK'$ Values of Fatty Acids in Ethanol-Water Mixtures—The $p$H of half neutralized 0.01 m solutions of the several fatty acids and sodium hydroxide containing varying amounts of ethanol was measured by means of the hydrogen electrode.

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<tr>
<th>Ethanol in ethanol-water mixture</th>
<th>Acetic acid</th>
<th>Proponic acid</th>
<th>Butyric acid</th>
<th>Caprylic acid</th>
<th>Lauric acid</th>
<th>Myristic acid</th>
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Measurements involving the higher fatty acids were, of course, obtainable only in higher concentrations of ethanol; precipitates were formed in lower concentrations, as shown in Table I.

It is evident from the data given in Table I that the higher fatty acids examined show remarkable similarity with regard to their $pK'$ values when measured in ethanol-water mixtures. This will be referred to under "Discussion."

Titration of Lysine and Arginine with Fatty Acids—Salts of weak bases with weak acids are subject to hydrolysis. The hydrolysis
constant may be obtained by subtracting the pK' value of the acid from the pK' value of the base. This method of evaluation eliminates a consideration of the pK' value of the solvent. The pK' values of lysine and arginine in 72 per cent ethanol are respectively 9.00 and 9.40 (2). The pK' values of lauric and oleic acids in the same solvent are respectively 6.83 and 6.84 (Table I). Hence the hydrolysis constant of the soaps of lysine and arginine with these respective fatty acids in 72 per cent ethanol will, in every case, be less than $10^{-2}$. The dissociation curves obtained by titration of lysine and arginine with lauric and oleic acids were calculated from the values computed for the hydrolysis constants by applying the equations of Kolthoff (3). The assumptions involved in the use of the equations are: (a) that the hydrogen and hydroxyl ion activities are approximately equal (at the point of equivalence of base and acid, the paH was calculated to be in the neighborhood of 8. The neutral point of the solvent used is probably about paH 7.7); (b) that hydrolysis is small.

Computed curves and observed values are shown in Fig. 1. The agreement is considered fairly satisfactory. The S-shape of the curves is indicative of salt formation.

**Titration of Salmine with Fatty Acids**—Electrometric titrations of a 0.82 per cent solution of salmine in 30 per cent by volume of ethanol with hydrochloric and caprylic acids in 30 per cent ethanol, and with hydrochloric and lauric acids in 72 per cent ethanol were carried out. The fatty acids readily neutralized the protamine but, due to the weakness of these acids, the titration curves obtained with them differed from the curves obtained with hydrochloric acid when extended to points where more than 1 equivalent of acid had been added. The equivalence of salmine as a base was calculated from the observed titration curve with caprylic acid in 30 per cent ethanol. Since this acid is so weak that it combines only with the excess basic groups of the salmine molecule (i.e., those groups which are not in zwitter ionic combination with carboxyl groups), the curve is not complicated by buffering with zwitter ions at low values of paH, which is the case in the titration curve with hydrochloric acid.

The pK' value of salmine was measured in various ethanol concentrations by obtaining its paH when half neutralized with hydrochloric acid. It was found to increase rapidly with the concentra-
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tion of ethanol, and, by extrapolation, to have a value of about 11.0 in water. Salmine is therefore a stronger base than arginine whose pK' value in water is 9.04 (4). These observations are thought to be of interest in connection with the structure of arginine (see "Discussion"). The results are shown in Table II and in Fig. 2.

 Preparation of Salts of Basic Amino Acids and Fatty Acids—Small samples of the following salts were prepared: lysine and arginine laurates, lysine and arginine stearates, arginine palmitate, and lysine erucate. The method used was to warm the pure amino acid with about 2 equivalents of fatty acid. The amino acid usually went into solution. A few drops of alcohol were added, followed by 10 volumes of ether. The salt was precipitated and the excess of fatty acid remained in solution. The precipitate was washed twice with ether. The salts possessed high melting points; for example, arginine stearate softened at 175° and melted with blackening at 185°. It was almost tasteless. In general, the salts were white soapy powders, insoluble in water.

![Fig. 1. Titration curves of lysine and arginine with lauric and oleic acids. The upper curve is calculated for the titration of arginine with lauric or oleic acids. The lower curve is calculated for the titration of lysine with lauric or oleic acids. The points represent experimental values. O, lysine and lauric acid; Δ, lysine and oleic acid; □, arginine and lauric acid; X, arginine and oleic acid.](http://www.jbc.org/)

Table II and Fig. 2.
TABLE II

<table>
<thead>
<tr>
<th>HCl per mole salmine, moles</th>
<th>0</th>
<th>0.29</th>
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<td>12.24</td>
<td>11.00</td>
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<th>Lauric acid per mole salmine, moles</th>
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<td>10.77</td>
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<td>pK'</td>
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<td>11.45</td>
<td>11.57</td>
<td>11.84</td>
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</table>

Fig. 2. Titration curves of salmine with hydrochloric acid (○) and with caprylic acid (×) in 30 per cent ethanol. The points indicate experimental observations, and the curves have been plotted to fit the points.
DISCUSSION

The influence of substituent groups upon the dissociation constant of the carboxyl group has been discussed in detail by Derick (5). It may be briefly summarized as follows: A substituent methyl group decreases the dissociation slightly. The effect will be greatly diminished by increasing its distance in the molecule from the carboxyl group. Therefore, methylacetic acid, or propionic acid, will be appreciably weaker than acetic acid. Lengthening of the chain by additional methylene groups will be practically without effect, because the distance of these groups from the carboxyl group is so great that they do not influence its dissociation.

This is nicely illustrated by the pK' values of fatty acids in 90 per cent ethanol listed in Table I. The similarity between the pK' values of all the higher fatty acids examined in ethanol-water mixtures strongly suggests that they have pK' values in water which lie between 4.9 to 5.0. This is in close agreement with the calculated value of 4.95 made by Derick (5) for caprylic acid.

Previous findings (2, 6) indicated that the pK' of an amino group was almost the same in ethanol-water mixtures as in water. An interesting departure from this rule was noted (2) in the case of the pK'_3 value of arginine, which was increased greatly by addition of ethanol. The pK'_3 value of arginine represents the guanidine group which carries the positive charge of the arginine zwitter ion. It has been pointed out (7) that the guanidine group probably ionizes in the resonant form

\[
\text{NH}_2 \quad \text{NH}_2 \quad \text{NH}_2
\]

\[
\text{H}_3\text{N}=-\text{C}=\text{NH} \quad \text{H}_3\text{N}=-\text{C}=\text{NH} \quad \text{H}_3\text{N}=-\text{C}=\text{NH}
\]

which accounts for its pronounced basicity. Hence the guanidine group may be expected to behave differently from an amino group since the former does not give rise to \(-\text{NH}_3\). This may explain its inertia in the nitrous acid and formaldehyde reactions, and the difference in the effect of ethanol on its pK' value from the effect of ethanol on the pK' value of an amino group. The data presented for the pK' values of salmine in ethanol-water mixtures furnish an interesting application of these observations. Since
Salmine contains 12 molecules of arginine for every 18 molecules of total amino acids (8), it is natural to assume that the basicity of salmine is due to free guanidine groups; the \( \alpha \)-amino groups of the arginine molecules are held probably in peptide linkage. The basic properties of free arginine are due principally to the \( \alpha \)-amino group, since the guanidine group exists in zwitter ionic combination with the carboxyl group. The \( pK' \) value of salmine is increased markedly by ethanol (Table II), while the \( pK'_{2} \) of arginine, which represents the \( \alpha \)-amino group, is not much changed by ethanol (2).

The properties of salmine may be expected to be cationic rather than zwitter ionic, since for every zwitter ion in its molecule, there must be at least twelve free basic groups.

It will be seen from Fig. 2 that the titration of salmine with caprylic acid in 30 per cent ethanol reached an end-point rather sharply. This was not the case with hydrochloric acid since buffering took place in the lower \( p\alpha H \) values which were not reached when caprylic acid was added.

**SUMMARY**

1. The \( pK' \) values of ten straight chain saturated fatty acids were measured in various ethanol-water mixtures. The values obtained indicated that all of the higher fatty acids examined possess nearly identical \( pK' \) values, and that their \( pK' \) values in water probably lie between 4.9 and 5.0.

2. Lysine and arginine were titrated electrometrically with lauric and oleic acids in 72 per cent ethanol. Fairly close agreement was obtained between the calculated and the observed values. The salts thus produced were hydrolyzed less than 1 per cent.

3. Titration curves of salmine with hydrochloric, caprylic, and lauric acids in ethanol-water mixtures indicate that combination took place in every instance.

4. The \( pK' \) value of salmine was found to be markedly increased by increasing the concentration of ethanol. Its value in water is about 11.0. Salmine is a stronger base than arginine.

5. Salts of lysine and arginine with certain of the higher fatty acids were prepared, and some of their properties are described briefly.
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BIBLIOGRAPHY

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