The Constitution of Ampholytes, Particularly That of Amino Acids, and Their Dissociation Constants

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1. Bredig[1] is probably the first to have called attention to the fact that an amphoteric electrolyte, and particularly betaine, is an internal salt, i. e. that the same molecule may contain a positive and a negative charge which neutralize each other. A few years later Küster[2] put forward a theory to explain the colour change of methyl orange. According to this theory methyl orange may exist in an electrically charged and yet electrically neutral form. For according to Küster yellow methyl orange is:

$$(CH_3)_2N.C_6H_4.N_2.C_6H_4.SO_3^-$$

and the red form, existing in acid solutions:

$$^+H(CH_3)_2N.C_6H_4.N_2.C_6H_4.SO_3^- \ .$$

Küster gives the name "Zwitterionen" ("ampho-ions") to "ions" like red methyl orange which carry a positive and a negative charge at the same time and consequently cannot contribute to the conduction of current. It is usually supposed that such forms of ions only exist in small quantities, and up to now the dissociation constants of amino acids have always been calculated on the assumption that these forms of ions are unimportant. Thus Walker[3] writes: "Die weitere Komplikation durch Annahme eines Zwitterions,

$$^+NH_3.R.COO^- \ ,$$

braucht nicht betrachtet zu werden, da bei dem gegenwärtigen Stand unserer Kenntnisse über amphotere Elektrolyte, diese Annahme nicht zur Erklärung der beobachteten Erscheinungen nötig ist." Michaelis[8] is even more emphatic in the new edition of his excellent book on concentration of hydrogen ions (p. 62). He writes of the amino acid ampho-ion: "Seine Menge ist zweifellos auch stets verschwindend klein", and later: "Wir haben bisher kein Mittel, die Existenz

der Zwitterionen nachzuweisen. Wegen ihrer stets sehr geringen Konzentration sind diese auch nicht imstande, das Gleichgewicht zwischen den anderen, bekannten Dissoziationsformen der Aminosäure messbar zu verschieben."

But as I shall try to show in the following, the situation is entirely the reverse. *For in all physiologically important amino acids the non-ionised (i.e. not as cations or anions existing) part of the dissolved matter occurs mainly as ampho-ions.* Only the dissociation constants which are calculated on the basis of this assumption will obtain really plausible values. Fortunately it does not matter whether the old or the new view is taken as a basis for calculating the equilibrium between cations, anions, and electrically neutral molecules. The important results obtained by Bredig, Walker, Michaelis and others in this field will still hold and only become clearer.

2. Küster arrived at his idea of the state of methyl orange in acid red solutions through the following considerations, which are still convincing. As all sulphonic acids are strong acids, the sulphonic group of methyl orange must always be strongly dissociated; the ionisation in the sulphonic group cannot, therefore, have anything to do with the colour change. Accordingly methyl orange must be supposed to act as an indicator because it contains an *amino* group. The colour changes when the latter group is turned into an electrically charged ammonium group by a hydrogen ion. This is also in accordance with the fact that methyl orange as an indicator is very similar to its mother substance dimethylamino-azo-benzene, which has no sulphonic group. The colours of both indicators are alike, and their colour change occurs in the same range of hydrogen-ion concentration (colour transformation range according to Sørensen[4] for methyl orange $p_H = 3.1 - 4.4$, for dimethylamino-azo-benzene $p_H = 2.9 - 4.0$). Thus according to Küster the red form of methyl orange is not:

$$(CH_3)_2N.C_6H_4.N_2.C_6H_4.SO_3H \ ,$$

but

$$^+HN(CH_3)_2.C_6H_4.N_2.C_6H_4.SO_3^- \ .$$

According to the views of organic chemists a quinoid transformation takes place giving:

$$^+(CH_3)_2N{:}C_6H_4{:}N.NH.C_6H_4.SO_3^- \ .$$

However, this is of no importance here.

Küster's train of thought seems very convincing. But it can only be applied to amino acids which are indicators, consequently for example not to amino-acetic acid. But as it is necessary in certain cases, as shown by Küster, to assume that the ampho-ion is the main form, it is likely that the same would occur in many other cases. And furthermore when it is remembered that 99.5 per cent of ammonium acetate in aqueous solution exists as:

$$NH_4^+ + CH_3COO^- ,$$

and only 0.5 per cent is hydrolysed into:

$$NH_3 + CH_3COOH ,$$

it seems simplest to assume that amino-acetic acid exists mainly in aqueous solution as:

$$^+NH_3.CH_2.COO^- .$$

We must expect the carboxyl group of amino-acetic acid to possess acidic properties, almost like those of acetic acid, and the amino group to possess basic properties, almost like those of ammonia. We shall, therefore, assume that the undissociated molecules of amino acids have a positive and a negative charge at the same time. On this assumption we shall calculate the dissociation constants of amino acids numerically.

3. According to the old view an amino acid $NH_2.R.COOH$ exists in aqueous solutions in three forms: as a cation $^+NH_3.R.COOH$, as an uncharged molecule $NH_2.R.COOH$, and as an anion $NH_2.R.COO^-$. The ratio between the three forms varies with the hydrogen-ion concentration of the solution. Let A^+, A, and A^- be the concentrations of these forms, and H^+ and OH^- the concentrations of the hydrogen ions and hydroxyl ions, respectively. The acidic dissociation constant k_a will then be defined by:

$$\frac{A^- \cdot H^+}{A} = k_a \qquad (1)$$

and the basic dissociation constant k_b by:

$$\frac{A^+ \cdot OH^-}{A} = k_b . \qquad (2)$$

According to the new view an amino acid in solution exists partly as the cation $^+NH_3.R.COOH$, and partly as the anion $NH_2.R.COO^-$ as above, but moreover as the ampho-ion $^+NH_3.R.COO^-$. We shall name the concentration of the latter form A^{+-}.* The dissociation of the carboxyl group then occurs according to the equation:

$$^+NH_3.R.COOH = {}^+NH_3.R.COO^- + H^+ ,$$

and for the corresponding acidic dissociation constant K_S we have:

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* In agreement with the hypothesis according to which neutral salts like NaCl, NH_4NO_3 are completely ionised, we shall not take into consideration the form:

$$\begin{array}{l} R.NH_3 \\ | \quad | \\ CO.O \end{array}$$

and in accordance with the predominant views we assume that the hydration of $—NH_2$ to $—NH_3.OH$ is slight.

$$\frac{A^{+-} \cdot H^+}{A^+} = K_S \,. \tag{3}$$

The dissociation of the amino group occurs according to:

$$H_2O + NH_2.R.COO^- = OH^- + {}^+NH_3.R.COO^-$$

and for the corresponding basic dissociation constant K_B we have:

$$\frac{A^{+-} \cdot OH^-}{A^-} = K_B \,. \tag{4}$$

If the old k_a- and k_b-values are known, it is easy to calculate the new dissociation constants, for A of the old exposition is equal to A^{+-} of the new; from the equations 1, 2, 3, and 4 we obtain:

$$K_S = K_{H_2O}/k_b \,, \qquad K_B = K_{H_2O}/k_a \,. \tag{5}$$

Here K_{H_2O} is the dissociation constant of water: $K_{H_2O} = H^+ \cdot OH^-$.

When an acid or a base has the dissociation constant K, it is well known that the hydrolysis constant of its salts is equal to K_{H_2O}/K. Thus it appears from equation 5 that the new dissociation constant K_S, which *characterizes the carboxyl group*, is identical with the hydrolysis constant which corresponds to the old basic dissociation constant k_b, and furthermore that the new dissociation constant K_B, which *characterizes the amino group*, is identical with the hydrolysis constant which corresponds to the old acidic dissociation constant k_a. By this change in the conception of the constitution of the amino acid molecule *the constant which formerly described the function of the amino group becomes a constant which characterizes the carboxyl group, and vice-versa.*

4. In table 1 the values of the old and new dissociation constants for a number of amino acids are collected, mainly according to the above-mentioned book by Michaelis. All constants are given as powers of 10 as this mode of writing is usually the most convenient. Actually it is sufficient to state the numerical value of the powers (—log K), for which I have previously proposed the term *dissociation exponent*[5]. To avoid misunderstandings the K-values themselves will, however, be stated in table 1. For the dissociation constant of water at 25° C the value $10^{-13.90}$ was used.

On converting the old k-value into the new K-value a difficulty is encountered in *lysine*, *arginine*, and *histidine*. The structural formulae of these substances show that they have one acidic function and two basic functions. Consequently it is not permissible to convert both old basicity constants into acidity constants. The first basicity constant has been transferred unchanged to the new system; the acidity constant was converted into the second basicity constant, and the second

basicity constant into the acidity constant. The following consideration has been guiding for this procedure:

It always appears *unambiguously* from the experimental data, at which hydrogen-ion concentrations (a_1, a_2, a_3...) the ampholyte possesses a buffer effect and changes its state of dissociation with the hydrogen-ion concentration. When the buffer effect at $H^+ = a$ is caused by an acidic group in the molecule, the acidity constant of this group is equal to a, and when it is caused by a basic group, the basicity constant equals $\frac{K_{H_2O}}{a}$. According to this principle the a-value can be calculated from the constants of the literature, and then it may be converted at pleasure into acidity or basicity constants.

Table 1. Dissociation Constants of Some Amino Acids at 25° C.

	k_a	k_b	K_S	K_B
Glycine	$10^{-9.75}$	$10^{-11.57}$	$10^{-2.33}$	$10^{-4.15}$
Methyl-glycine	$10^{-9.89}$	$10^{-11.75}$	$10^{-2.15}$	$10^{-4.01}$
Dimethyl-glycine	$10^{-9.85}$	$10^{-11.97}$	$10^{-1.93}$	$10^{-4.05}$
Betaine	abt. 10^{-14}	$10^{-12.66}$	$10^{-1.34}$	abt. 1
Alanine	$10^{-9.72}$	$10^{-11.29}$	$10^{-2.61}$	$10^{-4.18}$
Leucine	$10^{-9.75}$	$10^{-11.64}$	$10^{-2.26}$	$10^{-4.15}$
Phenylalanine	$10^{-8.60}$	$10^{-11.89}$	$10^{-2.01}$	$10^{-5.30}$
Tyrosine	$10^{-8.40}$	$10^{-11.39}$	$10^{-2.51}$	$10^{-5.50}$
Glycyl-glycine	$10^{-7.74}$	$10^{-10.70}$	$10^{-3.20}$	$10^{-6.16}$
Alanyl-glycine	$10^{-7.74}$	$10^{-10.70}$	$10^{-3.20}$	$10^{-6.16}$
Leucyl-glycine	$10^{-7.82}$	$10^{-10.52}$	$10^{-3.38}$	$10^{-6.08}$
Taurine	$10^{-8.8}$	abt. 10^{-14}	abt. 1	$10^{-5.1}$
Asparagine	$10^{-8.87}$	$10^{-11.82}$	$10^{-2.08}$	$10^{-5.03}$
Lysine { 1st stage dissociation	10^{-12}	$<10^{-6.96}$	$10^{-1.94}$	$10^{-1.9}$
Lysine { 2nd stage dissociation	—	$10^{-11.96}$	—	$10^{-6.96}$
Arginine { 1st stage dissociation	$<10^{-13.96}$	$10^{-7.0}$	$10^{-2.24}$	> 1
Arginine { 2nd stage dissociation	—	$10^{-11.66}$	—	$10^{-6.9}$
Histidine { 1st stage dissociation	$10^{-8.66}$	$10^{-8.24}$	$10^{-1.60}$	$10^{-5.24}$
Histidine { 2nd stage dissociation	—	$10^{-12.30}$	—	$10^{-8.24}$
Aspartic acid { 1st stage dissociation	$10^{-3.82}$	$10^{-11.92}$	$10^{-1.98}$	$10^{-1.8}$
Aspartic acid { 2nd stage dissociation	$10^{-12.1}$	—	$10^{-3.82}$	—

For *lysine* we find in the literature:

$$k_b \text{ (2nd stage dissociation)} = 10^{-11.96},$$
$$k_b \text{ (1st stage dissociation)} < 10^{-6.96},$$
$$k_a = 10^{-12}.$$

Hence:

$$a_1 = 10^{-1.94}, \qquad a_2 > 10^{-6.94}, \qquad a_3 = 10^{-12}.$$

The values are arranged according to falling hydrogen-ion concentrations. When it is assumed that the hydrogen ions of lysine are split off at these values in the following sequence:

$$\begin{array}{cccc} C_4H_8.NH_3^+ & C_4H_8.NH_3^+ & C_4H_8.NH_2 & C_4H_8.NH_2 \\ | & | & | & | \\ CH.NH_3^+ & \rightarrow CH.NH_3^+ & \rightarrow CH.NH_3^+ & \rightarrow CH.NH_2 \\ | & | & | & | \\ COOH & COO^- & COO^- & COO^- \ , \end{array}$$

the corresponding dissociation constants are:

$$K_S = 10^{-1.94} ,$$
$$K_B \text{ (2nd stage dissociation)} < 10^{-6.96} ,$$
$$K_B \text{ (1st stage dissociation)} = 10^{-1.9} .$$

For the tervalent ampholyte *aspartic acid* only two constants are given in the literature: an acidity constant k_a and a basicity constant k_b. The two a-values corresponding to these constants are $a_1 = 10^{-1.98}$ and $a_2 = 10^{-3.82}$. Winkelblech[6] has, however, published results which permit the calculation of the hydrogen-ion concentration for a third p_H-range. Winkelblech has measured the conductivity of aspartic acid + 2NaOH, but interpreted the result wrongly. By calculation from his measurements, I find k_a (2nd stage dissociation) $= 10^{-12.1}$, and so $a_3 = 10^{-12.1}$. When it is assumed that the hydrogen ions are split off from the cation of aspartic acid in the following sequence:

$$\begin{array}{cccc} COOH & COO^- & COO^- & COO^- \\ | & | & | & | \\ CH.NH_3^+ & \rightarrow CH.NH_3^+ & \rightarrow CH.NH_3^+ & \rightarrow CH.NH_2 \\ | & | & | & | \\ CH_2 & CH_2 & CH_2 & CH_2 \\ | & | & | & | \\ COOH & COOH & COO^- & COO^- \ , \end{array}$$

we obtain from the above three a-values:

$$K_S \text{ (1st stage dissociation)} = 10^{-1.98} ,$$
$$K_S \text{ (2nd stage dissociation)} = 10^{-3.82} ,$$
$$K_B = 10^{-1.8} .$$

5. We shall now consider the numerical values of the old and the new *acidity constants*. The acidity constants computed from the old k_a-values for most of the amino acids mentioned range from about 10^{-8} to 10^{-10}; arginine and betaine are still less, and only aspartic acid more acid. But these values are very improbable;

for all these acids with the exception of taurine are *carboxylic acids*, and most of the known carboxylic acids have acidity constants of the magnitude 10^{-2} to 10^{-5}. Taurine is a sulphonic acid, and as these acids are usually just as strong as sulphuric acid (k = about 1), the value ($10^{-8.8}$) found for taurine is also very strange. It has already been observed by Winkelblech[6] that the numerical values of k_a and k_b are hard to understand.

According to the new view the acidity constants get values from about $10^{-1.5}$ to $10^{-3.5}$. These values are certainly a little higher than those usually met with in carboxylic acids, but a certain increase in the acidic function of the carboxyl group is to be expected in these amino acids. When the dissociation scheme of acetic acid is compared with that of the carboxyl group of glycine

$$CH_3.COOH = CH_3.COO^- + H^+$$
$$^+NH_3.CH_2.COOH = {}^+NH_3.CH_2.COO^- + H^+ ,$$

it becomes at once obvious that the positively charged ammonium group of glycine must facilitate the dissociation of the hydrogen ion by its electric repulsion, and consequently increase the acidity of the carboxyl group. In the dibasic acids we have the opposite effect, namely that of a negative electrical charge. The second dissociation constant in these acids is usually from 10 to 10000 times smaller than the first. Comparing the two dissociation equations, e.g. of malonic acid:

$$COOH.CH_2.COOH = COOH.CH_2.COO^- + H^+$$
$$COOH.CH_2.COO^- = COO^-.CH_2.COO^- + H^+ ,$$

we see that the smallness of the second dissociation constant may be regarded as a consequence of the attractive effect of the first negatively charged carboxyl group on the hydrogen ion of the second.

It is interesting to see that the acidic function of an amino carboxylic acid is the stronger the nearer the carboxyl group is situated to the positively charged amino group, just as it was to be expected. For dipeptides, e.g. glycyl-glycine:

$$^+NH_3.CH_2.CO.NH.CH_2.COO^-$$

with its widely interspaced groups, K_S is approximately 10 times smaller than for the α-amino acids with neighbouring COOH- and NH_2-groups.

The explanation of the high value of the first dissociation constant in *aspartic acid* is as follows: At the dissociation of the first carboxyl group:

$$\begin{array}{l} {}^+NH_3.CH.COOH \\ \quad\quad | \\ \quad CH_2.COOH \end{array} = \begin{array}{l} {}^+NH_3.CH.COO^- + H^+ \\ \quad\quad | \\ \quad CH_2COOH \end{array}$$

we have to take into account not only the increment in acidic strength due to the positive charge of the ammonium group, but also the increment due to the second non-dissociated carboxyl group. That the carboxyl group in diamino-monocarboxylic acids like *lysine*, *arginine*, *histidine* is so strongly acidic ($K_S = 10^{-1.6}$ to $10^{-2.2}$) is due to the fact that in this case we have two positively charged ammonium groups, e.g. in lysine:

$$^{+}NH_3.C_4H_8.CHNH_3^{+}.COOH = {}^{+}NH_3.C_4H_8.CHNH_3^{+}.COO^{-} + H^{+}.$$

It agrees well with the sulphonic acid nature of *taurine* that it has a K_S of about 1.

The only unexpected thing is the increase in the acidic function of the carboxyl group through the introduction of methyl groups in the amino group of glycine.

6. Having discussed the advantages of the new view with regard to the acidity constants, we shall proceed to consider the *basicity constants*. The old k_b-value for most aliphatic amino acids lies between 10^{-10} and 10^{-12}; for taurine we have $k_b = 10^{-14}$. According to this the amino groups should be 10^6 to 10^{10} times less basic than ammonia and aliphatic amines, the dissociation constants of which are in the vicinity of 10^{-4}. Even betaine, which has a quaternary ammonium group, and arginine, which is a derivative of the alkali-like base *guanidine*, should thus have very weakly basic properties.

The situation becomes, however, quite clear when we consider the new K_B-values. For in this case the alkali-like character of the basic groups of *betaine* and *arginine* is expressed by the K_B-values being equal to 1 or even greater. *Glycine* and the other aliphatic α-amino acids have basicity constants from about 10^{-4} to 10^{-5}, that is about the same as ammonia and aliphatic amines. In *di-peptides*, e.g. glycyl-glycine:

$$H_2O + NH_2.CH_2.CO.NH.CH_2.COO^{-} = OH^{-} + {}^{+}NH_3.CH_2.CO.NH.CH_2.COO^{-}$$

the peptide binding evidently has a fairly strong electronegative* effect, because K_B is less than 10^{-6} for this substance. In *phenylalanine* and *tyrosine* it is the benzene nucleus which has an electronegative effect ($K_B = 10^{-5.3}$ and $10^{-5.5}$, respectively).

In *aspartic acid* K_B is exceptionally great (about $10^{-1.9}$). Here the dissociation scheme is:

$$H_2O + NH_2.CH(CH_2.COO^{-}).COO^{-} = OH^{-} + {}^{+}NH_3.CH(CH_2.COO^{-}).COO^{-}.$$

* "Electronegative" is used here as usual in chemistry in the meaning: increasing the acidic properties or decreasing the basic properties.

The two negatively charged carboxyl groups must facilitate the admission of a hydrogen ion into the amino group considerably and thereby make it more strongly basic. Also for *lysine* the first basicity constant is very great ($K_{B1} = 10^{-1.94}$). Its dissociation scheme is:

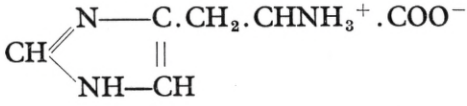
$$\begin{array}{l} C_4H_8.NH_2 \\ | \\ CH.NH_2 + H_2O \\ | \\ COO^- \end{array} = \begin{array}{l} C_4H_8.NH_2 \\ | \\ CH.NH_3^+ + OH^- \\ | \\ COO^- \ . \end{array}$$

Besides the negatively charged carboxyl group the terminal amino group also acts electropositively here and increases K_{B1}. Conversely at the ionisation of the second amino group:

$$\begin{array}{l} C_4H_8.NH_2 + H_2O \\ | \\ CH.NH_3^+ \\ | \\ COO^- \end{array} = \begin{array}{l} C_4H_8.NH_3^+ + OH^- \\ | \\ CH.NH_3^+ \\ | \\ COO^- \end{array}$$

the positively charged α-ammonium group must decrease the value of K_{B2} ($K_{B2} = 10^{-6.94}$). It should not surprise that the amino group has an electropositive, but the ammonium group an electronegative effect as a similar phenomenon is well known from the behaviour of dicarboxylic acids. For malonic acid for instance the first dissociation constant is greater and the second smaller than for acetic acid. Thus an undissociated carboxyl group has an electronegative effect while on the other hand an ionised carboxyl group has an electropositive effect.

That the second basicity constant of *histidine:*



is small ($10^{-8.24}$) was to be expected as the second basic function derives from the but weakly basic glyoxaline ring. That the first basicity constant is rather small, too, ($10^{-6.9}$) shows that the glyoxaline ring exerts a pronounced electronegative influence on the α-amino group.

7. On the whole it may be said that the assumption of the ampho-ion constitution results in dissociation constant values of the considered amino acids which conform excellently with their structural formulae, which was not the case with the old dissociation constants. From this it can be concluded that these amino acids must exist mainly as ampho-ions, just as Küster has already previously found it to be the case for methyl orange.

Why this conclusion was not drawn before can be explained as follows: It has been calculated that salts of acids and bases with dissociation constants like k_a and k_b should be almost completely hydrolysed, and consequently it was concluded that there could be no ampho-ions as they represent the non-hydrolysed salt form. But thereby it has been overlooked that the dissociation constants obtain quite different values when an ampho-ion constitution is assumed (cp. Walker[7], Winkelblech[6], and Michaelis[8]).

The aliphatic amino acids are *internal salts* of an acidic group with a basic group in the same molecule. They are closely related to a salt like ammonium acetate. But *acetates* having an alkaline reaction are not regarded as bases, and *ammonium salts* having an acidic reaction are not regarded as acids. Similarly *amino acids* should not be regarded as acids and bases at the same time. All these compounds are substances in which acidic and basic groups have interacted to form salts, and their respective acidic and alkaline reactions are to be explained by *hydrolysis*.

The old acidity constant of amino acids k_a is not the dissociation constant of a carboxyl group, but a hydrolysis constant corresponding to the amino group, and the former basicity constant k_b is not the dissociation constant of the amino group, but a hydrolysis constant corresponding to the carboxyl group.

When it is desired to calculate the influence of the hydrogen-ion concentration on the ratio between the quantities of free undissociated amino acid and its cation and anion, equations 1 and 2 with k_a and k_b may just as well be used as equations 3 and 4 with K_S and K_B. Mathematically the two systems are completely equivalent. The advantage of equations 3 and 4 and the constants K_S and K_B is only that in this system the values of the constants conform with the dissociation constants found for other carboxylic acids (respectively sulphonic acids) and amines. If we were used to express the strength of an acid by the hydrolysis constant of its sodium salt and the strength of a base by the hydrolysis constant of its chloride, it would also come most natural to use k_a and k_b, and not K_S and K_B, to indicate the acidic and basic functions of amino acids.

8. Already in the year 1867 Erlenmeyer has proposed a cyclic formula with quinquevalent nitrogen for *taurine*, and in 1875 he[9] has ascribed a similar cyclic formula to the amino carboxylic acids, anyway for the α-amino carboxylic acids.

Later on other scientists have also come to the conclusion of a cyclic formula of the amino acids from chemical evidence. An interesting work by Marckwald, Neumark and Stelzner[10] should especially be noted. In this work Marckwald concludes that as the amino acids of the fatty series do not react easily and smoothly with mustard oils, while all other primary amines usually react violently with mustard oils at ordinary temperature, amino acids contain no free amino group, but are salt-like substances of a cyclic constitution. He finds this view borne out

by the fact that even at ordinary temperature all amino acids investigated by him react upon all mustard oils in alkaline solution under generation of a considerable heat. For there is no doubt that they exist in alkaline solution as salts with the free amino group:

$$NH_2.R.COO.Na\ .$$

Translated into the terms of the ionic theory the cyclic formula becomes the ampho-ion formula of Bredig and Küster, and consequently we can regard Marckwald's work as an important argument for the ampho-ion nature of the aliphatic amines.

A certain corroboration of the ampho-ion formula is also derived from the behaviour of amino acids towards formaldehyde at Sørensen's "formol titration"[11]. At this titration the solution is first neutralised against litmus, then extra formaldehyde is added, and a titration with a base is performed with phenolphthalein as indicator until a deep red colour is obtained ($p_H > 9$). The consumption of base will be a measure of the amino groups present. By this method of determination formaldehyde reacts, forming methylene compounds with the amino groups, thus concealing the basic properties of the latter.

The aliphatic amino acids have two p_H-ranges in which they have a buffer effect. One is in the acid region at p_H = about 4, the other in the basic region at p_H = about 10. According to the old view which is expressed in the formula $NH_2.R.COOH$, the buffer effect is caused by the *amino group* at p_H = about 4, and by the *carboxyl group* at p_H = about 10. According to the new view, which assigns the formula $^+NH_3.R.COO^-$ to the free amino acids, it is exactly in the reverse. On addition of formaldehyde the buffer effect disappears at p_H = about 10; otherwise it would not have been possible to titrate to $p_H \lessapprox 9$ according to Sørensen and find a sufficiently sharp colour change. Consequently it comes more natural to assume that the amino group causes the buffer effect at p_H = about 10, and accordingly that the formula of the free amino acid is $^+NH_3.R.COO^-$.

The following consideration is even more convincing: Sørensen has shown that at the formol titration it is necessary to titrate to a strongly alkaline reaction because the binding of the amino group through formaldehyde only becomes complete in a strongly alkaline solution. He formulates the transformation for alanine:

$$\begin{array}{lllll} CH_3 & & CH_3 & & \\ | & & | & & \\ CH.NH_2 & + CH_2O & \rightleftarrows CH.N{:}CH_2 & + H_2O & \\ | & & | & & \\ COOH & + KOH & COOK & + H_2O & \end{array}$$

It is not immediately obvious from this scheme why the amino group only at strongly alkaline reaction is completely saturated by an excess of formaldehyde.

If there were a free amino group in the amino acid, one would expect the formation of a methylene compound to occur in neutral solution almost as completely as in alkaline solution. For potassium hydroxide does not attack the amino group as formaldehyde does, but reacts with another part of the molecule.

The behaviour of amino acids becomes, however, intelligible when the formula $^{+}NH_3.R.COO^{-}$ is assumed. According to this conception the free amino group does not appear until the amino acid in alkaline solution changes into the anion $NH_2.R.COO^{-}$. Before this release of the amino group has taken place the formation of the methylene compound cannot be complete.

The partial formation of the formaldehyde compound even without addition of base is probably to be explained by the presence of some $NH_2.R.COOH$ besides $^{+}NH_3.R.COO^{-}$.

9. Strictly speaking the solution of an amino acid contains $^{+}NH_3.R.COO^{-}$ as well as $NH_3.R.COOH$. What we have ascertained is only that the amphoion is by far the predominant form in the amino acids in question. It might be of interest to ascertain the numerical ratio between the quantities of these forms. According to the law of mass action this ratio is a constant for each amino acid in a diluted aqueous solution, irrespective of the other dissolved substances, particularly irrespective of the hydrogen-ion concentration.

In order to estimate the value of this constant from the dissociation constants we shall begin by assuming that the acidic character of the carboxyl group is not essentially dependent on the state of ionisation of the amino group. This assumption means that the acids:

$$^{+}NH_3.CH_2.COOH \quad \text{and} \quad NH_2.CH_2.COOH$$

should be almost equally strong. However, this will certainly only hold to a rough approximation, because the closer the amino group is situated to the carboxyl group, the more will the acidic function increase by the transformation of the amino group into a positively charged ammonium group. If K_1 is the dissociation constant of the acid $^{+}NH_3.CH_2.COOH$ and K_2 the dissociation constant of the acid $NH_2.CH_2.COOH$, we can write:

$$K_1 = n \cdot K_2 , \tag{6}$$

where n is a number between 1 and 10000. So far we are only able to estimate n roughly for the various amino acids.

If it is assumed that $NH_2.CH_2.COOH$ as well as $^{+}NH_3.CH_2.COO^{-}$ are to be found in the solution, the equations by which k_a, k_b, K_S, and K_B are determined must be written as follows

$$k_a = \frac{A^{-} \cdot H^{+}}{A + A^{+-}}, \tag{7}$$

$$k_b = \frac{A^+ \cdot OH^-}{A + A^{+-}}, \tag{8}$$

$$K_S = \frac{(A + A^{+-})\, H^+}{A^+}, \tag{9}$$

$$K_B = \frac{(A + A^{+-})\, OH^-}{A^-} \tag{10}$$

The ratio between the quantities of the two forms of the undissociated amino acid we shall name x:

$$x = \frac{A}{A + A^{+-}}, \qquad 1 - x = \frac{A^{+-}}{A + A^{+-}}. \tag{11}$$

For the dissociation constant K_1 of the acid $^+NH_3.CH_2.COOH$ we then obtain:

$$K_1 = \frac{A^{+-} \cdot H^+}{A^+} = (1 - x)K_S \tag{12}$$

and for the dissociation constant K_2 of the acid $NH_2.CH_2.COOH$:

$$K_2 = \frac{A^- \cdot H^+}{A} = \frac{A^- \cdot K_{H_2O}}{A \cdot OH^-} = \frac{K_{H_2O}}{x \cdot K_B}. \tag{13}$$

From equations 6, 12, and 13 we obtain:

$$x(1 - x) = n\frac{K_{H_2O}}{K_S K_B} \tag{14}$$

and by introducing k_a and k_b:

$$x(1 - x) = n \cdot \frac{k_a \cdot k_b}{K_{H_2O}}. \tag{15}$$

It is evident that equation 14 has no unambiguous solution. When a satisfies the equation, $1-a$ will do it, too. If for instance we find $x = 0.001$, $x = 0.999$ will also satisfy the equation. In order to decide between these two values, we must examine whether k_a and k_b or K_S and K_B are the better expressions for the acidic and basic properties of a substance of the constitution considered. Not till then can we decide whether 99.9 per cent occurs as $^+NH_3.R.COO^-$ or as $NH_2.R.COOH$.

Equation 15 affords an explanation of an observation by Michaelis[8] according to which ampholytes with $k_a.k_b$ equal to or greater than $K_{H_2O}(= 10^{-14})$ are not to be found in nature. According to equation 15 we have:

$$k_a \cdot k_b = \frac{x(1 - x)}{n} \cdot K_{H_2O}.$$

As $x(1-x)$ can at most become $\frac{1}{4}$, and n must be greater than 1 according to our theoretical molecular considerations, the product $k_a \cdot k_b$ can at most rise to $\frac{1}{4} K_{H_2O}$. Analogously it holds for $K_S \cdot K_B$ that it cannot be less than $4 K_{H_2O}$. In an α-amino acid in which the amino group and the carboxyl group are neighbours, and n consequently is large, $k_a \cdot k_b$ must be much less than $\frac{1}{4} K_{H_2O}$. In polypeptides and albumins amino groups and carboxyl groups may be situated so far from each other that they hardly influence each other. Here n will approach 1, and $k_a \cdot k_b$ may rise to $\frac{1}{4} K_{H_2O}$. But this does not mean that they must rise to that value.

The significance of the two identical expressions:

$$\frac{K_S \cdot K_B}{4K_{H_2O}} \quad \text{and} \quad \frac{K_{H_2O}}{4k_a \cdot k_b}$$

which are always greater than one, is illustrated by the following. Their square root gives the ratio at the isoelectric point between the concentrations of the free undissociated ampholyte $(A + A^{+-})$ and that part $(A^+ + A^-)$ which occurs as cation or anion. So neither this fraction can fall below 1. Consequently at least 50 per cent of the ampholytes are undissociated at the isoelectric point.

10. In table 2 the values of K_S, K_B, and $K_{H_2O}/(K_S \cdot K_B)$ are stated for some amino acids; furthermore the estimated values of n and the values of x calculated according to equation 14. It follows from our previous considerations that all the amino acids of the table occur mainly as $^+NH_3.R.COO^-$. The smaller of the two mathematically possible values should, therefore, be chosen.

Table 2

	K_S	K_B	$\frac{K_{H_2O}}{K_S K_B}$	n	x
Dimethyl-glycine	$10^{-1.93}$	$10^{-4.05}$	$10^{-7.92}$	10^4	$10^{-3.92}$
Glycine	$10^{-2.33}$	$10^{-4.15}$	$10^{-7.42}$	10^4	$10^{-3.42}$
Phenylalanine	$10^{-2.01}$	$10^{-5.30}$	$10^{-6.59}$	10^4	$10^{-2.59}$
Glycyl-glycine	$10^{-3.20}$	$10^{-6.16}$	$10^{-4.54}$	10^2	$10^{-2.54}$

In table 2 n is given the value 10^4 for α-amino acids, and 10^2 for dipeptides. These values were estimated as follows: By considering the dissociation constants of the dicarboxylic acids, particularly those of the symmetrical ones, we can observe the effect of a *negative* charge on the dissociation constants of a carboxyl group. In these acids the dissociating molecule is neutral at the dissociation of the first carboxyl group, and at the dissociation of the second group it is nega-

tively charged*. We may assume that the acidity constant of a carboxyl group will be increased approximately as much by the *positive* charging which accompanies the transition of an NH_2-group to a $^+NH_3$-group as it will be decreased by the *negative* charging which accompanies the transition of a COOH-group to a COO^--group. Now the ratio K_1/K_2 of the two acidity constants in dicarboxylic acids is never greater than 54000 (value for maleic acid). The ratio is 780 for oxalic acid, and only 25 for succinic acid. Accordingly we may be justified in regarding the n-value of table 2 as the upper limit. Even when using these high values for n, $^+NH_3.R.COO^-$ is found to prevail to such an extent that only 0.01 to 0.3 per cent of the form $NH_2.R.COOH$ is present.

When K_S or K_B *are approximately 1*, equation 14 may only be used with a certain qualification. In such cases we have to do with strong electrolytes the actual dissociation constants of which are much higher than those calculated according to the methods generally used up to now. Consequently the amphoion is more prevalent than it is calculated with the usual constants according to equation 14. Such conditions prevail in *betaine* and *taurine*. Indeed, the only possible form for betaine is $^+N(CH_3)_3.CH_2.COO^-$ as the other form $(CH_3)_2N.CH_2.COO.CH_3$, which is well known, shows properties quite different from those of betaine.

The preceding considerations were derived from an investigation of the *acidic function* of the compounds:

$$^+NH_3.R.COOH \quad \text{and} \quad NH_2.R.COOH.$$

We might, of course, just as well have considered the *basic function* of the compounds:

$$NH_2.R.COOH \quad \text{and} \quad NH_2.R.COO^-.$$

As we must arrive at the same result either way, we can conclude that the transition of the carboxyl group to the charged form will increase the basic function of the amino group to exactly the same degree as the acidic function of the carboxyl group is increased by the transition of the amino group to the charged form.

11. The behaviour of *aromatic* amino acids differs somewhat from that of the aliphatic amino acids. In table 3 the dissociation constants of some aromatic amino acids are collected. The k_a- and k_b-values of the *amino-benzoic acids* are of rather plausible magnitudes, which favour the formula $NH_2.C_6H_4.COOH$. For benzoic acid itself the dissociation constant is $10^{-4.22}$, and as the amino group acts in a positive direction, somewhat smaller constants are to be expected for carboxyl groups in the molecules $NH_2.C_6H_4COOH$. The k_a-values are from

* As will be shown in a following paper it is, however, only the decrease of K_2 below 1/4 K_1 which can be explained by the influence of charge.

Table 3. Dissociation Constants of Aromatic Amino Acids at 25° C.

	k_a	k_b	K_S	K_B
o-amino-benzoic acid	$10^{-4.98}$	$10^{-11.86}$	$10^{-2.04}$	$10^{-8.92}$
m- – – –	$10^{-4.92}$	$10^{-10.63}$	$10^{-3.27}$	$10^{-8.98}$
p- – – –	$10^{-4.80}$	$10^{-11.92}$	$10^{-1.98}$	$10^{-9.10}$
o-benz-betaine	$<10^{-14}$	$10^{-12.55}$	$10^{-1.35}$	$10^{0.1}$
m- – –	$<10^{-14}$	$10^{-10.47}$	$10^{-3.43}$	$10^{0.1}$
p- – –	abt. 10^{-14}	$10^{-10.49}$	$10^{-3.41}$	abt. $10^{0.1}$
o-amino-benzene sulphonic acid	$10^{-2.48}$	—	—	$10^{-12.42}$
m- – – – –	$10^{-3.73}$	—	—	$10^{-10.17}$
p- – – – –	$10^{-3.24}$	—	—	$10^{-10.64}$

$10^{-4.80}$ to $10^{-4.98}$, i.e. about 5 times smaller in actual fact. On the other hand the dissociation constant of aniline is $10^{-9.34}$, and as a carboxyl groups acts in a negative direction, the constants for the amino groups in the molecules $NH_2.C_6H_4.COOH$ must be smaller than $10^{-9.34}$. Actually the k_b-values are from 5 to 400 times smaller.

Not only k_a and k_b, but also K_S and K_B have, however, satisfactory values for these ampholytes. Consequently we cannot exclude the ampho-ion formula $^{+}NH_3.C_6H_4.\ COO^{-}$. K_S is from 10 to 200 times greater for all three amino-benzoic acids than for benzoic acid in accordance with the fact that the positively charged amino group increases the acidic function of the carboxyl group. Furthermore K_B for these compounds is about twice as great as for aniline. This agrees with the view that the negatively charged radical —COO^{-} increases the basic function of the amino group.

The preceding considerations lead to the result that amino-benzoic acids in aqueous solution probably occur in discernible quantities both as $NH_2.C_6H_4.COOH$ and as $^{+}NH_3.C_6H_4.COO^{-}$. If we calculate the ratio between the two forms according to equation 14, the same result is obtained. In this case $K_{H_2O}/(K_S \cdot K_B)$ has values between 1/45 and 1/910. If we write $n = 10$ to 100, we obtain about 0.1 to 0.9 for x. As n is not known with any accuracy x cannot be calculated more exactly. But it is fairly certain that the free amino-benzoic acids in aqueous solution consist of mixtures of $NH_2.C_6H_4.COOH$ and $^{+}NH_3.C_6H_4.COO^{-}$ with not less than 10 per cent of the smaller quantity.

It would be interesting to check this result by comparative investigations on the behaviour of the amino-benzoic acids and the aliphatic amino acids towards mustard oils and aldehydes.

With regard to the amino-benzoic acids it should moreover be pointed out that the maximum values for n can be obtained from equation 14, as $x(1—x)$ cannot exceed 1/4; n must consequently be smaller than:

$4 \cdot 10^{-2.94} = 1/294$ for *o*-amino-benzoic acid
$4 \cdot 10^{-1.65} = 1/11$ - *m*- - - -
$4 \cdot 10^{-2.82} = 1/166$ - *p*- - - -

The increasing influence of the transition from $—NH_2$ to $—NH_3^+$ on the acidic character of the carboxyl group (respectively the increasing influence of the transformation from —COOH into $—COO^-$ on the basic character of the amino group) is thus able to raise the value of the dissociation constants at most 11 times in the meta-compound, but in the para-compound 166 times, and in the ortho-compound 294 times. According to the current ideas of structure the greater effect is to be expected in the latter cases.

Table 3 shows that k_a and k_b for the *benz-betaines*:

$$^+(CH_3)_3N.C_6H_4.COO^-$$

are too small to permit of the conception that they contain free carboxyl groups and are quaternary ammonium bases. On the other hand the values of K_S($10^{-1.35}$ to $10^{-3.43}$) appear to go well with a carboxylic acid, the acidity of which has been increased through a positively charged ammonium group. Likewise the K_B-values, which are all greater than 1, agree well with the alkali-like character of quaternary ammonium bases. This leads to the constitution: $^+(CH_3)_3N.C_6H_4.COO^-$. Here the other possible constitution: $(CH_3)_2N.C_6H_4.COOCH_3$ can be excluded by chemical evidence, too. k_a and k_b or K_S and K_B could be used equally well for the amino-benzoic acids. For the benz-betaines, however, only K_S and K_B have values which agree with the structure of these substances.

In the same way only K_S and K_B are suited to represent the acidic and basic functions of the *amino-benzene sulphonic acids* quoted last in table 3. k_b has certainly not yet been measured, but we may assume that it is smaller than 10^{-14}, and consequently $K_S > 1$. The k_a-values are of the order of magnitude 10^{-3}, and as sulphonic acids are generally strong acids, it is improbable that these figures can characterize the acidity of a sulphonic acid group. On the other hand K_B (of the order of magnitude 10^{-11}) computed from these k_a-values may very well characterize the basicity of an aromatic amine. According to the above, the amino sulphonic acids occur exclusively in the ampho-ion form $^+NH_3.C_6H_4.SO_3^-$.

12. For all ampholytes considered so far K_S and K_B agree better with the structural formula than k_a and k_b, or at least as well. There are, however, ampholytes for which k_a and k_b are better suited to characterize their acidic and basic properties, e.g. *amino-phenols* with aromatic amino groups. In these substances the acidic and basic properties are only slightly pronounced with dissociation constants of the order of magnitude 10^{-10}. They will mainly exist in the undissociated state as $HO.R.NH_2$, and the acidity of the phenol group will be correctly represented by k_a, and the basicity of the amino group by k_b.

13. Now we are in a position to understand what K_S and K_B really express about the acidic and basic groups of an ampholyte. By transformation of equations 9 and 10 we get:

$$K_S = \frac{A^{+-} \cdot H^+}{A^+} + \frac{A \cdot H^+}{A^+}; \qquad K_B = \frac{A^{+-} \cdot OH^-}{A^-} + \frac{A \cdot OH^-}{A^-}.$$

We see that K_S is the sum of *a dissociation constant* of the acid $^+NH_3.R.COOH$ and *a hydrolysis constant* of the salt of the amine $NH_2.R.COOH$. Analogously K_B is the sum of the dissociation constant of the amine $NH_2.R.COO^-$ and the hydrolysis constant of the salt of the acid $^+NH_3.R.COOH$. If the dissociation constants are much greater than the hydrolysis constants, K_S will be a dissociation constant of the carboxyl group, and K_B one of the amino group. This holds for the aliphatic amino acids. When, conversely, the hydrolysis constants are much greater than the dissociation constants, the latter may be ignored in the sums. In this case (e.g. in the amino-phenols) K_S becomes a hydrolysis constant characteristic of the amino group, and K_B one characteristic of the phenol group. In the aromatic amino acids the dissociation constants and the hydrolysis constants are of the same order of magnitude. So here we cannot decipher the acidic nature of the carboxyl group and the basic nature of the amino group separately from K_S and K_B.

Similar considerations can be made on k_a and k_b, only that it is here the reciprocal values: $1/k_a$ and $1/k_b$ which must be taken as sums of reciprocal dissociation and hydrolysis constants.

14. Now the question arises whether it is possible to determine the ratio A^{+-}/A in any other way, too, so as to check and extend the result obtained. This question must be answered in the affirmative, and in the following I shall outline some courses possible for this purpose.

If the ionisation of the basic or the acidic group in a coloured ampholyte is connected with a colour change, there is the possibility of a quantitative colorimetric determination. When for instance in the case of an amino acid the formation of the $^+NH_3$-group causes a colour change, while the dissociation of the carboxyl group does not change the colour, the two forms of the undissociated ampholytes will have different colours. The *ampho-ion* form will have the same colour as the cation $^+NH_3.R.COOH$, and the *amino acid form proper* $NH_2.R.COOH$ will have the same colour as the *anion* $NH_2.R.COO^-$. The light absorption of the *cation* is measurable in a strongly acid solution, and that of the *anion* in a strongly basic solution. The light absorption of the *undissociated ampholytes* can be ascertained by an absorption measurement at a hydrogen-ion concentration at which an appreciable fraction of the ampholyte is undissociated. The more the light absorption of the undissociated ampholyte approaches that of the cation, the more

predominant is the ampho-ion. On the other hand $NH_2.R.COOH$ is predominant when the absorption approaches that of the anion. If E_K, E_A, and E_U are the extinction coefficients of the cation, the anion, and the undissociated ampholyte at a certain wave-length,

$$\frac{A^{+-}}{A^{+-} + A} = \frac{E_U - E_A}{E_K - E_A}$$

will hold.

We shall apply this method to *methyl red* (*p*-dimethylamino-azo-benzene-*o*-carboxylic acid). In this case we may expect both forms to be present in quantities of the same order of magnitude as in amino-benzoic acids. This is necessary for ascertaining the ratio of their quantities according to the above method.

15. A second possibility for the determination of A^{+-}/A is based on the following consideration. We may expect the solubility of the form of $NH_2.R.COOH$ not to be influenced to a higher degree by the addition of neutral salt than the solubilities of non-electrolytes in general. But it is different with the form of $^{+}NH_2.R.COOH^{-}$. On account of its electrical charges an *addition of neutral salt must be expected to decrease its activity and increase its solubility* as it is always the case with salts without common ions. When the two charges are very far from each other, the increase should be twice that of an ordinary univalent salt. The closer the charges are to each other, the smaller must be the effect, the charges mutually weakening each other.

Provisional solubility determinations of *methyl orange* in potassium chloride solutions made in this laboratory by Max Møller have given an effect which is $^1/_3$ of that estimated from the assumption that the distance between the charges is great. According to these experiments the solubility of methyl orange in 0.01 normal hydrochloric acid is increased by potassium chloride in the following way:

Table 4

Solvent	Solubility at 25° C.	c	k
0.01 normal HCl	$0.379 \cdot 10^{-4}$ molar	0.01	—
0.01 normal HCl, 0.05 normal KCl	$0.408 \cdot 10^{-4}$ –	0.06	0.108
0.01 normal HCl, 0.5 normal KCl	$0.501 \cdot 10^{-4}$ –	0.51	0.105
0.01 normal HCl, 1.75 normal KCl	$0.520 \cdot 10^{-4}$ –	1.76	0.068

The solubilities were determined colorimetrically.

In 0.01 normal HCl methyl orange exists in the red ampho-ion form except for a few per cent. The solid substance was a form of the free deeply red-violet methyl orange crystallizing in needles into which the freshly precipitated form was transformed spontaneously when shaken with water.

For the activity coefficient f of a univalent ion in an aqueous solution of univalent ions of the normality c we may write approximatively[12]:

$$-\log f = k \sqrt[3]{c}$$

and then find values for k *(the activity constant)* near 0.3. For methyl orange we shall write:

$$-\log f = 2 \cdot k \sqrt[3]{c}\,.$$

When s_1 and s_2 are the solubilities at the ion normalities c_1 and c_2, and f_1 and f_2 are the corresponding activity coefficients, we have:

$$\log s_2 - \log s_1 = \log f_1 - \log f_2 = 2k\,(\sqrt[3]{c_2} - \sqrt[3]{c_1})$$

and hence:

$$k = \frac{\log s_2 - \log s_1}{2(\sqrt[3]{c_2} - \sqrt[3]{c_1})}$$

In table 4, c stands for the ionic concentration (i.e. the concentration of ionised electrolyte) and k for the activity constants calculated by means of the above equation. Excluding the value in 1.75 normal KCl, because the cube root formula cannot be expected to hold even approximately in this case, we find the mean $k = 0.107$, that is somewhat more than $^1/_3$ of 0.3, the mean value for univalent ions.

The ampho-ion of an ampholyte must also, like all ions, decrease the activity of other ions present although less strongly because the charges partly neutralize each other.

In order to ascertain this, Carl Faurholt has at my request measured the solubility of croceo cobaltic nitrate $(NO_2)_2Co(NH_3)_4.NO_3$ in different salts and in *glycine* at 18° C and found:

in water	0.0118 moles per litre
in 0.1 normal sodium chlorate	0.0145 - - - *
in 0.1 normal sodium formate	0.0142 - - -
in 0.1 normal glycine	0.0127 - - -

The solubilities were determined by decomposition of the croceo complex with sodium hydroxide and titration of the ammonia distilled off. It appears from the measurements that the effect of glycine is 9/25 of the effect of ordinary univalent salts.

Also for the *activity decreasing* effect in glycine we thus find somewhat over 1/3 of the effect to be expected when it is assumed that the distance between the charges is great.

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* The solution was supersaturated with croceo cobaltic chlorate.

Mixtures of amino acids are much more easily soluble in water than might be expected from the solubilities of the individual acids. Not only do the acids crystallize with greater difficulty, but they are actually more soluble. This increase in solubility which is unexpected in view of the slight solubility of these substances can be explained by their ampho-ionic nature, viz. by the attractive forces between the electrical charges.

It will be of interest to determine the decrease of activity and increase of solubility of the various *amino-benzoic acids* and *amino-benzene sulphonic acids*, respectively, by addition of neutral salts. In this way we can in the first place estimate the distances between the ortho, meta and para-positions. The already well known action of the radicals in *o*, *m*, and *p*-position on each other, e.g. the intensifying effect of a nitro group on the acidic character of benzoic acid is presumably rather due to the length of the atom chain than to the distance in space. Secondly we shall be able to calculate the quantity of the ampho-ion form in *o*-(respectively *m*, *p*) amino-benzoic acid by comparing this acid with *o*-(respectively *m*, *p*) amino-benzene sulphonic acid, because the neutral salt effect must be approximately the same for the ampho-ion form of the two acids, and we know that the amino sulphonic acids exist exclusively in the ampho-ion form. From the experimentally accessible and undoubtedly smaller salt effect in solutions of amino-benzoic acids we may, therefore, draw conclusions as to the quantity of the form $NH_2.C_6H_4.COOH$.

16. It is probable that ampholytes which mainly occur in solution as ampho-ions also have this constitution in the solid state. The molecules must then be held together in their crystal lattices by strong electrical forces, being situated according to something like the following scheme:

$$\begin{array}{lll} {}^{+}NH_3.R.COO^{-} & {}^{+}NH_3.R.COO^{-} & {}^{+}NH_3.R.COO^{-} \\ {}^{-}OOC.R.NH_3{}^{+} & {}^{-}OOC.R.NH_3{}^{+} & {}^{-}OOC.R.NH_3{}^{+} \\ {}^{+}NH_3.R.COO^{-} & {}^{+}NH_3.R.COO^{-} & {}^{+}NH_3.R.COO^{-} \end{array}$$

Besides the usual cohesion forces we have in this case strong electrical forces. We must expect such substances to be slightly soluble, particularly in organic solvents which do not, owing to great dielectric constants, facilitate the dissociation of the electrically charged molecules from each other. In accordance with this the ordinary amino acids are actually almost insoluble in ether and alcohol, and only soluble to some extent in water, which has a large dielectric constant. Also the comparatively high melting point of many amino acids can be explained by the firm binding of the molecules in the crystals.

In Meyer and Jacobson's textbook of organic chemistry[13] these physical properties are quoted as arguments for the cyclic formula with quinquevalent nitrogen.

A crystal structure, like the one described above, will probably *tend towards*

the formation of electrically charged particles when pulverized, the molecules being fissured occasionally, thereby causing particles with positive and negative charges to arise. In amino acids with neighbouring positive and negative charges such a fission is not, however, very probable. All the same it is known that occasionally amino acids may become strongly electrical when pulverized. In polypeptides a fission may occur more easily. Actually the observation has been made by N. Troensegaard[14] that, in certain derivatives from albumins, the substance became so strongly electrical when pulverized that the loose powder could hardly be removed from mortar and pestle. Perhaps we may regard this as evidence in favour of an ampho-ion constitution. In the ordinary ion lattices such phenomena will not be expected, because an electrical charge may easily be neutralised by transition of ions, and moreover the electrical conductivity is not sufficiently small.

Many amino acids may be sublimed, i.e. they may exist in vapour form. According to Willstätter's[15] investigations they evaporate in the form of $NH_2.R.COOH$. At any rate Willstätter has shown that by heating above the boiling point in a closed tube *betaines* turn into amino esters, e.g. $(CH_3)_2N.CH_2.COO.CH_3$.

SUMMARY

1. In their undissociated state the *aliphatic amino acids* exist almost exclusively (to the extent of more than 99.5 per cent) as salt-like *ampho-ions* $^{+}NH_3.R.COO^{-}$. Thus they are not amino acids proper, but *ammonium salts*. A content of hydrogen or hydroxyl ions in their solutions does not mean that they are acids or bases, but only that they are hydrolysed as salts.

The constants k_a and k_b by means of which the acidic and basic properties of the amino acids have hitherto been indicated are not dissociation constants, they are *hydrolysis constants*. The true *dissociation constants* which indicate the strengths of the neutralized acidic and basic groups in the amino acid molecule are:

$$K_S = K_{H_2O}/k_b \text{ and } K_B = K_{H_2O}/k_a$$

(K_{H_2O} is the dissociation constant of water). K_S and K_B have values for the amino acids which agree with their structural formulae. The values of K_S and K_B may be estimated from the nature of the acidic and basic group, when we take into account the influence of the other substituents in the usual way.

Various chemical reactions confirm that these substances contain *no free amino group*. Their physical properties agree with their salt-like nature. Their behaviour is also salt-like in that they increase the solubility of other salts and are themselves more easily soluble in salt solutions than in water.

2. In the aromatic amino acids *ampho-ions*, $^{+}NH_3.R.COO^{-}$, as well as *amino acid molecules proper*, $NH_2.R.COOH$, with a free amino group and carboxyl group are present (in quantities of from 10 to 90 per cent).

3. In the aromatic amino phenols the ampho-ion form is completely suppressed.

4. From the magnitude of the dissociation constant of an *ampholyte* it is possible to estimate the ratio between the quantities of the salt-like ampho-ion and the amino acid proper.

5. It is shown theoretically that probably $K_S\ K_B$ for an *ampholyte* can never be smaller than 4 K_{H_2O} ($k_a\ k_b$ never greater than $^1/_4 K_{H_2O}$). It follows from this that the proportion of an ampholyte existing as cation and anion can never amount to more than 50 per cent.

6. It is suggested how the results obtained may be checked by investigations of colours and solubilities. Preliminary experiments of this kind are quoted.

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