occurred (pH 2.7). Small as this depression is, it corresponds to $\log K' = 11.0$ at this part of the titration. Glutamic acid gave a depression of only 0.07 pH unit. It is becoming clear that the avidity of substances for ferric salts can be measured only if there is no interfering pK_a between 1.5 and 3, or if the avidity is so great that basic ferric salts are not precipitated at pH 3.

Concluding remarks. In spite of the highly individual mode of union with metallic ions shown by many of the amino-acids examined here, no departure has been found from Mellor & Maley's order of avidity, discussed in Part 1. A different order, however, exists among the pteridines, the purines and riboflavine as will be shown in Part 3.

The amino-acids showed no departure from simple stepwise addition. The values for $\log K' - \log K''$ tend to lie between 1 and 2 (type (II) complexes tend to have higher values than types (I) or (III); the values for copper and nickel tend to be higher than those of the other metals, but are not proportional to $\log K_s$).

SUMMARY

1. The avidities of α -amino-acids with three ionizing groups for the ions of heavy metals have been measured and recorded as stability constants.

For any one metal the stability constants varied over an enormous range (e.g. by 10^{11} for nickel). However, the order of avidities, for any given amino-acid, remains as with glycine (see Table 3).

2. Tyrosine, ornithine, lysine, arginine, aspartic acid and glutamic acid were found to combine through the α -amino- and α -carboxyl-groups, after the manner of glycine. Other acids combine through the α - and the ω -nitrogen atoms (or, in the case of cysteine, the sulphur atom). The homologous series: lysine, ornithine, $\alpha\gamma$ -diaminobutyric acid and $\alpha\beta$ -diaminopropionic acid has been examined and found to yield examples of transition between these two extremes (see Tables 2 and 3).

3. Several of the acids show abnormalities of behaviour with copper; cysteine and histidine show anomalies with cobalt.

4. The metal-binding properties of histamine have been studied for comparison with those of histidine.

5. The ionization constants of $\alpha\gamma$ -diaminobutyric, $\alpha\beta$ -diaminopropionic and benzoylglutamic acids have been determined for the first time.

The author wishes to thank Mr J. N. Phillips and Prof. W. O. Kermack, F.R.S., for most valuable theoretical discussions; also Mr E. P. Serjeant, who carried out the majority of the titrations.

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APPENDIX

Derivation of Equations

By J. N. PHILLIPS

A. Derivation of equation for $\bar{n}$

In the simple case where only two ionizing groups are present, the total concentration of complexing agent $[XSc^0]$ is the sum of five concentrations:

$$[XSc^0] = [Sc] + [ScH^+] + [ScH_2^{++}] + [ScM] + 2[Sc_2M]. \quad (\text{viii})$$

But, by definition, $\bar{n}$ is the average number of molecules of complexing agent bound per atom of the metal, i.e.

$$\bar{n} = \frac{[ScM] + 2[Sc_2M]}{[M^0]}. \quad (\text{ix})$$

Hence

$$\bar{n}[M^0] = [ScM] + 2[Sc_2M]. \quad (\text{x})$$

Substituting (x) into (viii) gives

$$[\text{XSc}^\circ] = [\text{Sc}] + [\text{ScH}^+] + [\text{ScH}_2^{++}] + \bar{n} [\text{M}^\circ]. \quad (\text{xi})$$

Now, in the Mass Action equations (xii),

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}, \text{ and } K'_a = \frac{[\text{H}^+][\text{HA}]}{[\text{H}_2\text{A}^+]}, \quad (\text{xii})$$

which describe the ionization of a dibasic acid, $[\text{A}^-]$ corresponds to $[\text{Sc}]$, $[\text{HA}]$ to $[\text{ScH}^+]$, etc. Hence

$$[\text{ScH}_2^{++}] = \frac{[\text{H}^+]^2 [\text{Sc}]}{K_a K'_a} \text{ and } [\text{ScH}^+] = \frac{[\text{H}^+] [\text{Sc}]}{K_a}. \quad (\text{xiii})$$

Substituting (xiii) into (xi) gives

$$[\text{XSc}^\circ] = [\text{Sc}] + \frac{[\text{H}^+]^2 [\text{Sc}]}{K_a K'_a} + \frac{[\text{H}^+] [\text{Sc}]}{K_a} + \bar{n} [\text{M}^\circ], \quad (\text{xiv})$$

rearrangement of which gives

$$\bar{n} = \frac{[\text{XSc}^\circ] - [\text{Sc}] \left\{ \frac{[\text{H}^+]^2}{K_a K'_a} + \frac{[\text{H}^+]}{K_a} + 1 \right\}}{[\text{M}^\circ]} \quad (\text{xv})$$

$$= \frac{[\text{XSc}^\circ] - \alpha [\text{Sc}]}{[\text{M}^\circ]}. \quad (\text{xvi})$$

Where three ionizing groups are present, the term

$$\frac{[\text{H}^+]^3}{K K'_a K''_a}$$

is added to α .

B. Derivation of equation for $[\text{Sc}]$

The concentration of free and combined hydrogen ions $[\text{XH}^+]$ can be expressed in two ways, (xvii) and (xviii):

$$[\text{XH}^+] = [\text{H}^+] + [\text{ScH}^+] + 2 [\text{ScH}_2^{++}] - [\text{OH}^-], \quad (\text{xvii})$$

$$[\text{XH}^+] = [\text{XSc}^\circ] + [\text{HCl}] - [\text{KOH}], \quad (\text{xviii})$$

where $[\text{HCl}]$ and $[\text{KOH}]$ equal the concentrations which these reagents would reach if run into the same volume of pure water. Combining (xvii) and (xviii),

$$2 [\text{ScH}_2^{++}] + [\text{ScH}^+] = [\text{XSc}^\circ] + [\text{HCl}] - [\text{KOH}] - [\text{H}^+] + [\text{OH}^-]. \quad (\text{xix})$$

Substituting terms from equation (xiii), and rearranging,

$$[\text{Sc}] = \frac{[\text{XSc}^\circ] + [\text{HCl}] - [\text{KOH}] - [\text{H}^+] + [\text{OH}^-]}{2 \frac{[\text{H}^+]^2}{K_a K'_a} + \frac{[\text{H}^+]}{K_a}} \quad (\text{xx})$$

$$= \frac{[\text{XSc}^\circ] + [\text{HCl}] - [\text{KOH}] - [\text{H}^+] + [\text{OH}^-]}{\beta}. \quad (\text{xxi})$$

$[\text{HCl}]$ and $[\text{KOH}]$ are alternative terms, depending on which reagent was used in the titration. In general $[\text{OH}^-]$ becomes significant only above pH 10. Where three ionizing

groups are present, the term $\frac{3 [\text{H}^+]^3}{K_a K'_a K''_a}$ is added to β .

Photometric Micro-analytical Estimation of Acetic Acid in Blood and Tissues

BY E. CIARANFI AND A. FONNESU

Institute of General Pathology, University of Perugia, Italy

(Received 23 February 1951)

The chemical methods which have been proposed for the estimation of acetic acid in biological materials are not specific and are, in consequence, of restricted value. Treatment of acetic acid with cupric oxide yields oxalic acid, and this reaction was utilized by Mugdan & Wimmer (1933) for the estimation of acetic acid; but, under their conditions tartaric, glycollic and the lower fatty acids also yield oxalic acid. Pyrolysis of calcium acetate yields acetone, but since the calcium salts of other monocarboxylic acids also yield carbonyl compounds under these conditions, the method of Dulian (1939), which is based on this reaction, is inadequate. A photometric method based on the lanthanum reaction of Kruger & Tschirch (1929) has recently been developed by Hutchens & Kass (1949), but since propionic acid also gives the blue colour their method lacks specificity.

Procedures dependent on the physical properties of acetic acid can be classified into: (i) Distillation methods: Duclaux (1900), Dyer (1917), Olmsted, Whitaker & Duden (1929), McClendon (1944) and

Black (1949). (ii) Partition methods: Behrens (1926), Werkman (1930*a, b, c*, 1931). Friedemann (1938) devised a method which is a combination of both the distillation and partition methods. (iii) Chromatographic methods: Ramsey & Patterson (1945, 1948), Elsdon (1946), Ramsey (1948), Moyle, Baldwin & Scarisbrick (1948) and Peterson & Johnson (1948). Of all these procedures only those involving chromatography can make any real claim either to specificity or to precision.

Acetic acid, however, does differ from the other members of the homologous series in its stability to the potassium dichromate-sulphuric acid reagent; but at the same time it is completely oxidized by treating it with a silver dichromate-sulphuric acid mixture (Simon, 1922*a, b*). Cordebard & Michl (1928) used this difference in behaviour as the basis of a quantitative method for the estimation of acetic acid in known mixtures; their method was adapted to the micro-estimation of acetic acid by Caselli & Ciaranfi (1942). In this procedure acetic acid was separated from its higher homologues by conversion