# XXVIII. THE TITRATION CONSTANTS OF ANSERINE, CARNOSINE AND SOME RELATED COMPOUNDS

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ALTHOUGH carnosine and anserine are characteristic constituents of the muscles of vertebrates, and are present in these tissues in amounts comparable with the adenylpyrophosphate and creatinephosphate contents, little is known of their chemical properties, and nothing of their physiological purpose. The present communication is primarily a contribution to our knowledge of their chemistry, though the results seem to have a bearing on the question of their function in muscle. As part of a chemical examination of these compounds we have measured their titration curves, together with those of methyl histidine, histidine, alanine and $\beta$-alanine. Certain of these measurements have been made already, but we wished to consider the group of related substances as a whole, and therefore thought it desirable to make measurements ourselves in these cases.

## EXPERIMENTAL

*Substances used:*

*Alanine*, Merck's "pure chemical".

*$\beta$-Alanine*, synthesized from succinimide [Organic Syntheses, 1936] recrystallized five times from hot dilute alcohol and dried *in vacuo* over $P_2O_5$ at 100°; M.P. 201° (decomp.).

l-*Histidine hydrochloride*, $C_6H_9O_2N_3HCl$, $H_2O$ (B.D.H.), recrystallized from water and dried *in vacuo* over $P_2O_5$; M.P. 255° (decomp.), $[\alpha]_D^{20°}$ +2·0° in water ($c$=3·5, 1 dm. tube).

l-*Methylhistidine*, prepared from anserine by acid hydrolysis [Linneweh & Linneweh, 1930], recrystallized three times from water and dried *in vacuo* over $P_2O_5$ at 130°; M.P. 247° (decomp.), $[\alpha]_D^{21°}$ −28·2° in water ($c$=1·38, 1 dm. tube). (Found: C, 49·3; H, 6·5; N, 24·7; (N)$CH_3$, 9·5%. $C_7H_{11}O_2N_3$ requires C, 49·7; H, 6·5; N, 24·85; (N)$CH_3$, 8·9%.)

*Carnosine nitrate*, isolated from horse muscle [Deutsch *et al.*, 1938] in the form of crystalline copper salt, which after three recrystallizations was converted into the nitrate, recrystallized three times from hot dilute alcohol and dried *in vacuo* over $P_2O_5$ at 100°; M.P. 227° (decomp.), $[\alpha]_D^{20°}$ +23·6° in water ($c$=3·5, 1 dm. tube). (Found: C, 37·3; H, 5·3; N, 24·0; (N)$CH_3$, 0·0%. $C_9H_{14}O_3N_4.HNO_3$ requires C, 37·35; H, 5·2; N, 24·2; (N)$CH_3$, 0·0%.)

*Anserine nitrate*, isolated from rabbit muscle [Deutsch *et al.*, 1938], in the form of crystalline copper salt, which after three recrystallizations was converted into the nitrate, recrystallized three times from hot dilute alcohol and dried *in vacuo* over $P_2O_5$ at 100°; M.P. 226° (decomp.), $[\alpha]_D^{20}$ +14·0° and +12·8° in water ($c$=3·74 and 6·5, 1 dm. tube). (Found: C, 39·7; H, 5·7; N, 23·4; (N)$CH_3$, 5·0%. $C_{10}H_{16}O_3N_4.HNO_3$ requires C, 39·6; H, 5·65; N, 23·1; (N)$CH_3$, 4·95%.)

*Procedure:*

Electrometric titrations were made in an air-chest thermo-regulated to 22°. A hydrogen electrode of the Cole pattern was used, the half-cell being connected by a saturated KCl-agar bridge to an approximately 4·5 *N* calomel half-cell. The E.M.F. of the cell was measured to the nearest 0·5 mV. by means of a Cambridge portable potentiometer. The potentiometer was standardized frequently by reference to a Weston standard cell (kept in the air-chest) of which the E.M.F. was taken to be 1018·5 mV. The hydrogen used was generated electrolytically from KOH solution between nickel electrodes and was passed over heated copper. It was therefore saturated with water vapour, but was presumed free from all other impurities. The E.M.F. readings were corrected to a barometric pressure of 760 mm. by means of the table given by Clark [1928].

The procedure adopted was to weigh 1 millimol. or rather less of the substance under examination, dissolve it in 10 ml. distilled water, and titrate it with an exactly normal solution of HCl or of NaOH, as required. The HCl was prepared from a constant boiling distillate, and its accuracy counterchecked against pure NaCl by electrometric titration with $AgNO_3$. The NaOH was prepared from a carbonate-free saturated solution, and was standardized against the HCl.

The E.M.F. corresponding to the $pK'$ value of any single titrating group was calculated from each experimental point on its titration curve by means of the generally accepted approximate relationship

$$E_{pK'} = E - 58{\cdot}5 \log \left(\frac{T}{t} - 1\right),$$

where $T$ is the end-point titre, $t$ the titre corresponding to the E.M.F. $E$, and 58·5 is the value of the conversion factor appropriate to a temperature of 22°. In the case of a group titrating in markedly acid or alkaline conditions the values of $t$ were corrected by the amount of acid (or alkali) needed to establish the same potential in an equal volume of pure water.

Finally the value of $pK'$ was calculated from the mean value of $E_{pK'}$ by the use of the expression

$$pK' = \frac{\text{mean value of } E_{pK'} - 249{\cdot}5}{58{\cdot}5},$$

in which the quantity 249·5 is the E.M.F. of the calomel half-cell referred to the normal hydrogen electrode. The E.M.F. of the cell when completed with *N*/10 HCl was 312·5 mV.; with *M*/5 acetate buffer, 519·5 mV.; with *M*/15 phthalate buffer, 482 mV.; with *N*/10 NaOH, 1005 mV. These figures lead to the following values for the *p*H of these solutions: *N*/10 HCl, 1·078; acetate buffer, 4·62; phthalate buffer, 3·98; *N*/10 NaOH, 12·91.

## Results

The values found for the titration constants of the substances examined are assembled in Table I. No attempt is made in the table to allocate these constants to particular titratable groups; the symbol $pK'$ implies the *p*H about which one titration curve is centred, under the conditions specified. It is however known in the cases of glycine, alanine and histidine that the group titrating in strongly acid solution is the carboxyl group, and that titrating in the region of *p*H 9–10 is the amino group. The second constant of histidine ($pK'$ 6·15) has been shown by Levy [1935] to belong to the iminazole group. The constants of methylhistidine may be assigned with confidence, since each differs so little from the corresponding constant of histidine. In carnosine the acid group of the β-alanine and the amino group of the histidine have been destroyed, and it is therefore probable that $pK_1'$ refers to the histidine carboxyl group, $pK_2'$ to the

Table I. *Titration constants of carnosine, anserine and some related compounds*

| Experiment | | | Constants | | |
|---|---|---|---|---|---|
| Substance | Concentration mols./kg. water | Temp. ° C. | $pK_1'$ | $pK_2'$ | $pK_3'$ |
| Glycine | 0·10 | 19·3 | 2·42 | — | — |
| | 0·10 | 22 | — | 9·82 | — |
| Alanine | 0·10 | 18·6 | 2·40 | — | — |
| β-Alanine | 0·10 | 18·6 | 3·63 | — | — |
| | 0·10 | 22 | 3·59 | — | — |
| | 0·10 | 22 | — | 10·3 | — |
| Histidine hydrochloride | 0·096 | 22 | — | 6·13 | 9·32 |
| | 0·05 | 22 | 1·69 | — | — |
| | 0·095 | 22 | — | 6·17 | 9·28 |
| *N*-Methylhistidine | 0·07 | 22 | 1·69 | 6·48 | — |
| | 0·07 | 22 | — | — | 8·85 |
| Carnosine nitrate | 0·03 | 22 | — | 6·78 | 9·48 |
| | 0·068 | 22 | — | 6·88 | 9·54 |
| | 0·065 | 22 | 2·65 | — | — |
| | 0·067 | 22 | 2·63 | — | — |
| Anserine nitrate | 0·03 | 22 | — | 7·00 | 9·45 |
| | 0·063 | 22 | 2·65 | — | — |
| | 0·067 | 22 | — | 7·08 | 9·53 |
| | 0·065 | 22 | 2·64 | — | — |

iminazole group, and $pK_3'$ to the amino group of the β-alanine residue. The constants of anserine may be similarly assigned.

Two facts emerge which are of interest to the physiologist. One is that there is no serious difference between anserine and carnosine in their titration curves, the other is that both substances are buffers over the physiologically important range of *p*H 6–8. The amount of these substances in the muscles of vertebrates (about 0·1 *M*) is such that their buffering action should be a significant fraction of the total buffering power of the tissue in the region of *p*H 7. It is doubtful, however, whether this is their primary function in the muscles. If it is, an explanation must be sought elsewhere of the existence of both anserine and carnosine in one and the same muscle, since they do not differ significantly in the property in question.

SUMMARY

1. The titration constants of methylhistidine, carnosine and anserine have been measured with a hydrogen electrode at 22° at concentrations specified in the text.

2. Carnosine and anserine differ very little in their apparent dissociation constants.

3. Carnosine and anserine are both buffers over the range *p*H 6–8.

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