CIV. THE POTENTIOMETRIC TITRATION OF SOLUTIONS OF VITAMIN B₁.

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(Received February 28th, 1935.)

THIS work forms part of a programme designed to throw light upon the constitution of vitamin B₁ prepared in this laboratory [Kinnersley *et al.*, 1935]; an unavoidable delay in starting gave us the advantage of having in our hands the results of a titration of Jansen's vitamin, privately communicated by Birch and Harris [Birch, 1934], when they wrote for a specimen of our vitamin to compare its behaviour with their previous results. We understand that they have this work in hand.

They obtained preliminary evidence of a dissociation constant at p_K 5.1 and another in the neighbourhood of p_K 9; that part of the titration curve representing the p_K 9 group was characterised by the fact that, on addition of acid or alkali, equilibrium was not immediately attained; a large change of p_H was produced immediately after each addition, but the p_H drifted back to more alkaline or acid values respectively. In the course of a complete titration from p_H 3 to 11 and back, about 20 % of the p_K 9 group was destroyed, the amount of the p_K 5.1 group remaining unchanged.

The properties of the p_K 9 group suggest at once those of a pseudo-acid converted to the acid form, or of a base converted to the pseudo-base form, as p_H is increased. It was desired if possible to determine the nature of this group. If this hypothesis is correct, the equilibrium may be represented, for the case of a pseudo-acid as

The overall dissociation constant K is given by

$$K = \frac{K_1 K_2}{1 + K_1} \quad \text{.....(B).}$$

It is apparent that any method for determining the nature of the dissociating group which relies on measuring the difference of K in two media, is not directly applicable here, since the effect of change of medium on K_1 is not measurable or predictable, so that the change of K_2 cannot be determined. It has however been found possible to determine K_2 independently of K_1 in water and 90 % alcohol.

EXPERIMENTAL.

The technique of these determinations is to be described elsewhere. The titration cells were constructed to hold a solution of 0.25 mg. in 2 ml. of solvent; the liquid junctions were made in sintered plugs of finely ground glass and were found to be reproducible to within 2 mv. Titrant solutions of strength $N/10$ were added from capillary micro-burettes. The p_H scales were standardised as

described by Ogston and Brown [1935]; the glass membranes used with a Macfarlane-Pye valve potentiometer were standardised with buffer solutions checked against the hydrogen electrode. Stirring of the solutions used with the glass electrode was effected by bubbling nitrogen slowly through them. Blank experiments showed that the temperature of the room remained sufficiently constant for the accuracy required ($18 \pm 0.5^\circ$), and that any absorption of carbon dioxide from the air, even by alkaline solutions, was negligible.

The dissociation constants were calculated from the experimental values by the method described by Ogston and Brown [1935].

RESULTS.

Experiments were conducted both at 18° and at 0° using hydrogen and glass electrodes in aqueous solutions and glass electrodes in 90 % ethyl alcoholic solutions. The form of curve obtained by Birch and Harris was confirmed, with certain minor differences. The results of various experiments may be summarised as follows.

I. Using the hydrogen electrode, neutral solutions of the vitamin dihydrochloride in water at 18° were titrated with acid to p_H 3, then with alkali up to p_H 10 and again back to p_H 3 with acid.

(1) Between p_H 3 and 7 the curve can be described by two constants of p_K 2.5 and 4.7; on the other hand the initial curve obtained on first titrating to p_H 3 was never reproduced in subsequent titration of the same solution (Fig. 1).

Fig. 1. Titration with hydrogen electrode at 18° (uncorrected). Curve I. Initial titration with acid. Curve II. Acid part of titration with alkali. Curve III. Return curve from p_H 10, with acid, showing shift to alkaline side.

(2) Between p_H 7 and p_H 10 the drifting of p_H after additions of titrant was confirmed; the time taken from each addition of titrant till equilibrium was established was about $\frac{3}{4}$ hour. Four such "equilibrium" curves gave values of p_K between 8.2 and 8.5, the mean being 8.3.

(3) Displacement of the curve as a whole to the alkaline side was observed on titrating back with acid, without change of its form, *i.e.* without alteration of the amount or dissociation constant of any of the groups titrated (Fig. 1). No disappearance of the p_K 8.3 group occurred.

II. Similar "equilibrium" titrations were carried out using glass electrodes in aqueous and in 90 % alcoholic solutions at 18° . The constants obtained in water were p_K 3.4, 4.8 and 9.1; in 90 % alcohol p_K 4.2 and 9.3 (values for the group having p_K 3.4 in water were not obtained since titration was not

carried to a low enough p_H). The general behaviour was the same as with the hydrogen electrode with the following differences.

(1) The initial curve obtained by titrating with acid to p_H 3 (*cf.* I (1) above) was exactly reproduced on titrating back with alkali.

(2) The constants of two of the groups were different.

(3) The displacement of the curves as a whole (I (3) above) was not observed.

In 90 % alcoholic solutions the behaviour is similar to that in water except that in alkaline solution 2–3 hours are required for establishment of equilibrium after each addition of titrant.

III. Assuming the molecule of the vitamin to be $C_{12}H_{16}ON_4S \cdot 2HCl$, the amounts of the two basic groups were within 5 % of 1 equivalent per mol. The amount of the p_K 9 group was, using the hydrogen electrode, sometimes 1, sometimes 2 equivalents per mol.; using the glass electrode, always 2 equivalents per mol.

IV. Assuming the pseudo-acid or pseudo-base hypothesis to be correct, the slowness with which equilibrium is established should make it possible to titrate the vitamin in each form separately by performing rapid titrations. This was done using the glass and hydrogen electrodes; in order further to retard the attainment of equilibrium, the cell was immersed in melting ice (see Fig. 2 which illustrates the form of the curves obtained).

Fig. 2. Rapid titrations with the hydrogen electrode at 0° (uncorrected). Curve I. Rapid titration with alkali (identical with solvent blank). Curve II. Rapid titration with acid after establishment of equilibrium.

(1) Solutions of the vitamin dihydrochloride were titrated rapidly with alkali to p_H 11; the results were the same using either hydrogen or glass electrodes and showed the absence of any group having a p_K greater than 7 (*i.e.* the curves were identical with the water blank).

(2) Solutions of the vitamin were brought rapidly to p_H 11 with alkali and allowed to stand until equilibrium was established (as before, the p_H fell steadily during this process); they were then rapidly titrated back with acid. The resulting curves gave constants p_K 6.8 in water, and p_K 7.9 in 90 % alcohol. With the hydrogen electrode a p_K of 6.4 was obtained (*cf.* II (2)).

V. The colour reaction test was performed on the residual solutions from all runs; where the hydrogen electrode had been used the reaction showed only 5–10 % of the strength expected from the amount of vitamin in solution; where

glass electrodes had been used, the colour reaction showed about 30 % of the expected value.

The constants obtained are summarised in the table.

Table I.

	p_K			Δp_K (glass) H ₂ O - 90 % EtOH
	H ₂ . Pt in H ₂ O	Glass in H ₂ O	Glass in 90 % EtOH	
Basic group 1. 18°	2.5	3.4	—	—
Basic group 2. 18°	4.7	4.8	4.2	-0.6
Acid group. Equilibrium	8.3	9.1	9.3	+0.2
p_K 18°				
p_{K_2} 0°	6.4	6.8	7.9	+1.1

DISCUSSION.

There is a marked difference of behaviour of the vitamin solutions with hydrogen and glass electrodes; with glass, their behaviour is more reproducible, but apart from this the main differences are:

(1) The difference between p_K 2.5 and p_K 3.4 for the weaker basic group, together with the anomaly of the initial part of the curve (I (1) and Curve I); both these can be accounted for by conversion of this group by action of the hydrogen electrode into one having a lower p_K .

(2) The difference found in the colour reaction (V).

(3) The displacement of the whole curve towards the alkaline side (I (3)).

(4) The difference between p_K 8.3 and 9.1 for the constant in this region.

(5) The difference between the amounts of the p_K 8-9 group, using hydrogen and glass electrodes (IV).

Some of these anomalies are unexplained, but in view of the present scarcity of material it was thought that further work was not justified. However, it seems likely that the vitamin is reduced by the hydrogen electrode, so that the results from the glass electrode best represent its behaviour: the group concerned in the colour reaction can be none of those titrated.

It was found that the vitamin does not poison the hydrogen electrode, nor does it appear to be strongly adsorbed.

The two acid-binding groups of the vitamin have p_K values of 3.4 and 4.8 respectively; this is in accord with the chemical data on the formation of hydrochlorides and sulphates [Kinnersley *et al.*, 1935]. The change of the second from p_K 4.8 to 4.2 between water and 90 % alcohol is of the order to be expected of a basic nitrogen atom [Neuberger, 1934]. Levene and Sims [1925] and Levene *et al.* [1926] have shown that pyrimidine amino-groups have p_K values of about this amount (4.0-4.6), so that the group of p_K 4.8 might be identified with the pyrimidine amino-group of Williams's [1935] formula. However, the quaternary nitrogen demanded by his formula is in disagreement with the evidence, since this must be a third, strongly basic group which together with the groups of p_K 3.4 and 4.8 would cause the vitamin to combine with three equivalents of acid instead of the two equivalents observed.

It appears that the vitamin has also a pseudo-acid group, in view of the following facts.

(1) Apart from the two basic groups (p_K 3.4 and 4.8) the form of the vitamin stable in acid solution contains no acid or basic groups titrating between p_H 7 and 11 (IV (1) above).

(2) Alkali causes the reversible formation of a group having p_K 6.8 in water and 7.9 in 90 % alcohol (IV (2) above). This change of p_K is characteristic of an enolic acid (unpublished observations of one of the authors).

This might be ascribed to a keto-enol change but for the fact that there appear two equivalents of acid per mol. of vitamin. The splitting of an oxide ring or of a sulphide ring (as in Williams's formula) might account for the facts, provided that the dissociation constants of the two —OH groups, or of the —OH and —SH so formed, were not very different. The possibility of hydrolysis of a —CO—NH— linkage seems to be excluded. The formation of a pseudo-base from quaternary nitrogen, such as that found in nicotine derivatives by Lowry and Lloyd [1932], will not account for the present results, both in view of the amount of the group (two equivalents per mol.), and in view of the change of p_K found between water and 90 % alcohol.

The titration results can be correlated with the absorption spectra of the vitamin [Peters and Philpot, 1933; Holiday, 1935] on the assumption that the labile band observed on bringing the vitamin from acid solution to p_H 9.5 is due to the unionised pseudo-acid form; as conversion of PA (equation A) to HA and A^- occurs, this band disappears.

SUMMARY.

Potentiometric titrations have been performed on solutions of crystalline vitamin B_1 dihydrochloride. Evidence has been obtained of the existence of two weakly basic groups of p_K 3.4 and 4.8, and of a pseudo-acid group titrating in the region of p_H 9. The dissociation constant of the acid form of the vitamin has the value p_K 6.8 in water at 0°.

The authors wish to express their thanks to Mr Kinnersley and to Mr Philpot for help and advice; and to Prof. Peters, at whose suggestion this work was undertaken, for invaluable advice throughout its course and for measurement of the colour reactions.

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