T H E S I S

on

THE IONIZATION CONSTANT OF PANTOTHENIC ACID

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THE IONIZATION CONSTANT OF PANTOTHENIC ACID

Introduction:

This work was undertaken in order to throw some light on the molecular structure of "pantothenic acid", the yeast growth stimulant studied by Williams and his associates.[1] Previous work has indicated that it is an alcohol-acid with two or more hydroxyl groups; and probably contains no element other than carbon, hydrogen, and oxygen. Its molecular weight is about 150, as determined by studies on the rate of diffusion through sintered glass diaphragms, and into gelatin. These specifications limit the probable number of carbon atoms in the molecule to five or six. As the ionization constant of an alcohol-acid depends on the position of the hydroxyl groups with respect to the carboxyl group in the molecule, it was hoped that this investigation involving a study of the acidic strength of the compound would give indications as to the location of the hydroxyl groups.

Method Proposed:

Since pantothenic acid has never been prepared in pure form, the usual methods of determining ionization constants could not, of course, be applied. In the present work, a method was utilized for obtaining an approximation, which, so far as we know, has never been applied before.

This work has its origin in the work done by R.R. Williams and Waterman[2] wherein they found that in a solution of a mixture of ampholytes, when electrolyzed in an apparatus consisting of several cells, each ampholyte migrates from both ends of the system to the region or cell where the pH corresponds to the isoelectric point of the individual ampholyte. By this means, it is possible to separate substances which otherwise would be very difficult, or impossible, to separate.

This method was first used successfully by Williams and Truesdail[3] in connection with the "bios" of Wildiers. Using an apparatus of four cells, connected by siphons, they were able to effect a sharp resolution of "bios" into two components, one acidic in reaction, and one basic.

Subsequently, Williams and his associates[1] electrolyzed extracts from various types of organisms, using a similar apparatus of eight cells. In every case, there was a very marked concentration of the yeast growth stimulant at pH 3.6 - 4.0. This was at first taken to indicate that the growth stimulant was an ampholyte whose isoelectric point was 3.6 - 4.0. Later experiments, in which the solution, before electrolysis, was slightly acidified, however, failed to show any migration toward

the basic end of the system, as one would expect in the case of an ampholyte. The behavior of this substance in becoming concentrated at a pH of about 3.8, without, however, migrating from the more acid end of the system, is in accord with what should be expected of a weak, non-amphoteric acid. The absence of basic groups in the molecule is further indicated by the tendency of the methyl and ethyl esters of pantothenic acid to migrate toward the acid end of the system, and by the regular variation of the adsorption on fuller's earth with pH from pH 6.5 to pH 0.9.

From the expression defining the ionization constant of an acid (1), we may derive equation (2),

$$K = \frac{[H^+][An^-]}{M - [An^-]} \qquad (1)$$

$$\frac{[An^-]}{M} = \frac{K}{K + [H^+]} \qquad (2)$$

in which, M is the molality of the undissociated acid, and the other symbols have their customary significance. Thus it is seen that the degree of ionization of a weak acid , at a given temperature is dependent only on the hydrogen ion concentration of the solution, and the ionization constant of the acid. It is also evident that the degree of ionization decreases with increasing hydrogen ion concentration, and that as an acid migrates toward the acid end of the system, it will eventually encounter

a region where its own ionization is repressed to such an extent that it will practically cease to migrate.

The region out of which an acid completely migrates, as is clear from the above discussion, depends intirely upon the ionization constant of the acid, and not upon its concentration in the solution. The fact that pantothenic acid is always completely removed from cells with a pH of 5 or higher indicates that it has a definite, though hitherto unknown, ionization constant.

The method proposed in this work, therefore, is to determine under like conditions of electrolysis, the points of acidity from which various acids of known ionization constants are removed. By comparing these values with those obtained for pantothenic acid, an approximate value for the ionization constant of this unknown acid may be ascertained.

Experimental Procedure:

The apparatus used included a system of eight glass cells or cups, each of about 65 cc. capacity, connected by siphons which could be simultaneously filled or emptied. Platinum electrodes were used. In most of the runs, one half of the secondary of a 15,000 volt transformer, and a rectifier tube were used. (See Fig. 1) In some of the preliminary runs, a 1,500 D.C. generator was used.

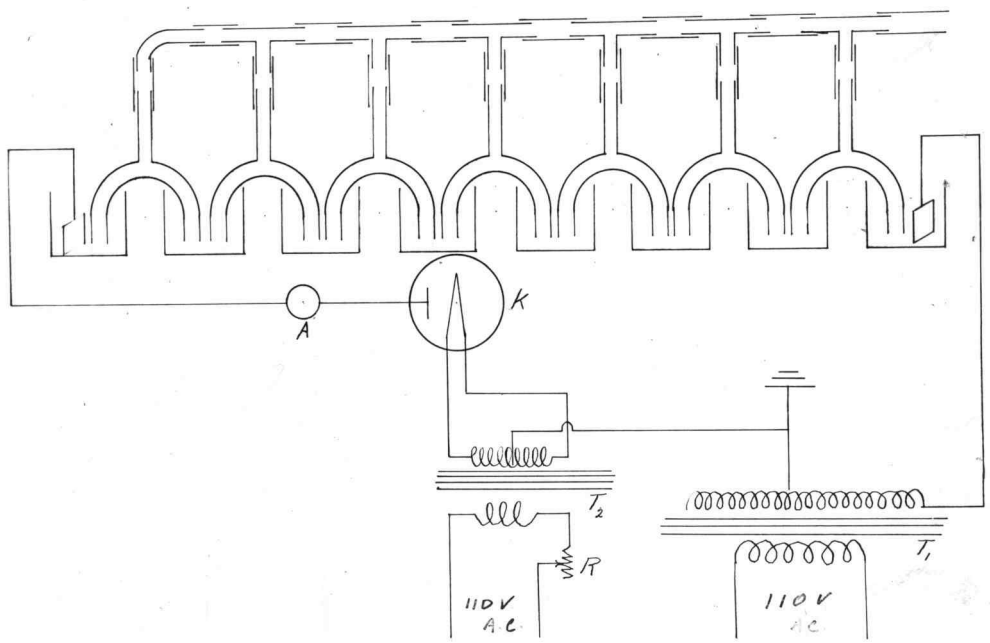

Fig. 1

In Fig.1, the symbols have the following designations;

T_1 ----- G.E. Luminous Tube Transformer Type YVU 2447A3

T_2 ----- Cenco F3865C Transformer

K ------ G.E. Kenotron FP-85

R ------ Variable resistance, up to 250 ohms

A ------ Milliammeter

The electrolytic solutions contained 50 mg. of 60% methanol extract of rice bran, dialyzed to remove colloidal material, and varying amounts of an acid of known ionization constant, dissolved in 425 cc. of water. To this solution was added 0.7 cc. of a phosphoric acid solution made by adding 1 cc. of syrupy phosphoric acid to 100 cc. of water. The phosphoric acid increases the conductivity

of thesolution, and affords a better pH gradient at the conclusion of the electrolysis. The rice bran extract contains the unknown pantothenic acid.

After electrolyzing for the desired length of time, the pH of the contents of each cell was determined by means of the quinhydrone electrode, and the amounts of both pantothenic acid and the acid of known ionization constant in each cell were estimated.

The method of analysis for pantothenic acid consists intesting the ability of the solution in question to stimulate the growth of Gebrude Mayer yeast. Three parallel tests of the contents of each cell after electrolysis were made as follows; 2 cc. of the solution were introduced into a 50 cc. Erlenmeyer flask, 1 cc. into a second flask, and 0.5 cc. into a third. Sufficient water was added to the second and third flasks to bring the volume of sulution in them up to 2 cc. For purposes of comparison, standards, containing, respectively, 0.474, 0.344, 0.236, and 0.118 mg. of unelectrolyzed rice bran extract; and two blanks containing only distilled water were prepared. After sterilization, each of the above flasks was seeded with 10 cc. of medium in which was suspended 0.004 mg. of moist yeast. After 18 hours' incubation in the flasks, without agitation at 30°C, the growth was measured with the apparatus originated by Williams, McAlister and

Roehm[4]. By plotting the amounts of extract in the standards against the corresponding galvanometer deflections, a curve was obtained from which the relative amounts of pantothenic acid in a series of unknowns could be determined with a fair degree of accuracy from the corresponding galvanometer readings. In the tests made, the yeast was grown on a medium containing the following ingredients;

1. Sucrose	20	gr.
2. $(NH_4)_2SO_4$	3	"
3. KH_2PO_4	2	"
4. Asparagin	1.5	"
5. $CaCl_2 \cdot 2H_2O$	0.25	"
6. $MgSO_4 \cdot 7H_2O$	0.25	"
7. Inositol	0.005	"
8. $TlCl_3$	0.001	"
9. $ZnSO_4$	0.001	"
10. $MnCl_2$	0.001	"
11. H_3BO_3	0.001	"
12. $CuSO_4$	0.0001	"
13. KI	0.0001	"
14. $FeCl_2$	0.0005	"
15. Water	1000	cc.

Constituents 1-7 of the medium contain all the known chemical substances which we have any reason to believe would be favorable to the growth of yeast. The remaining

constituents (8-14) are added only in traces, and their positive effect is dubious. These were used for our own satisfaction in view of the results of others who attribute importance to these substances.

In the proximate analysis for gallic acid, advantage was taken of the formation of a colored compound upon rendering a solution of gallic acid alkaline.[5] Samples were taken from each cell and made alkaline. The colors of the resulting solutions were compared by means of Nessler tubes, with standards containing known amounts of gallic acid, treated with the same amount of alkali.

As pyrogallol forms a colored compound with alkali, the analytical procedure used for it was the same as that used for gallic acid.

In the determination of salicylic acid, the solution to be analyzed was made acid, if not already so, and extracted with ether. The ether solution was extracted with dilute alkali, the extract neutralized, and treated with ferric alum solution. By the use of Nessler tubes, the resulting color was compared with those of standards containing known amounts of salicylic acid, similarly treated.[6]

The analyses for caproic acid and benzoic acid were accomplished by extracting the solution to be analyzed with ether, adding a known excess of 0.01 N. alkali to the ether solution, evaporating off the ether, taking up the

residue with water, and back titrating with 0.01 N. acid. As there was acid present in the original solution, several blank runs were made to determine the distribution of the original acid after electrolysis, and the corrections to be applied to the acid analyses of the various cells. As the pH gradients were not identical for any two runs, the corrections varied accordingly. Since the corrections so varied, the results with caproic acid and benzoic acid were only rough approximations.

TABLE I

Results of Electrolysis

Acid added ---------- Gallic acid

Amount ---------- 2.5 mg.

Time of electrolysis ------ 24 hours

Voltage ---------- 7,500 (half wave)

Cell No.	pH of cell after electrolysis	Percentage of total gallic acid in cell	Percentage of total panthenic acid in cell		
			Test 1 (2 cc.)	Test 2 (1 cc.)	Test 3 (0.5 cc.)
1	2.75	5.5	7.7	4.6	5.2
2	3.45	30.6	32.2	28.3	30.8
3	3.85	55.3	45.5	53.9	53.9
4	4.55	6.5	14.5	13.2	10.2
5	5.25	0.0	0	0	0
6	5.35	0.0	0	0	0
7	5.00	0.0	0	0	0
8	9.00	0.0	0	0	0

TABLE II

Results of Electrolysis

Acid added ---------- Gallic acid

Amount ---------- 5 mg.

Time of electrolysis ----- 24 hours

Voltage ---------- 7,500 (half wave)

Cell No.	pH of cell after electrolysis	Percentage of total gallic acid in cell	Percentage of total pantothenic acid in cell	
			Test 1 (1cc.)	Test 2 (0.5 cc.)
1	3.00	22.2	11.9	11.1
2	3.85	44.0	40.4	50.1
3	4.40	36.4	48.2	38.9
4	5.05	0.0	0	0
5	5.40	0.0	0	0
6	5.20	0.0	0	0
7	5.30	0.0	0	0
8	8.20	0.0	0	0

TABLE III

Results of Electrolysis

Acid added ---------- Gallic acid

Amount ---------- 10 mg.

Time of electrolysis ------- 24 hours

Voltage ---------- 7,500 (half wave)

Cell No.	pH of cell after electrolysis	Percentage of total gallic acid in cell	Percentage of total pantothenic acid in cell		
			Test 1 (2 cc.)	Test 2 (1 cc.)	Test 3 (0.5 cc)
1	3.55	16.7	9.5	13.4	14.9
2	3.65	32.2	31.0	28.4	29.5
3	4.50	37.9	36.5	37.6	37.7
4	4.75	13.1	23.1	20.8	18.0
5	5.30	0.0	0	0	0
6	4.35	0.0	0	0	0
7	5.30	0.0	0	0	0
8	7.50	0.0	0	0	0

TABLE IV

Results of Electrolysis

Acid added ---------- Gallic acid

Amount ---------- 5 mg.

Time of electrolysis ------ 18 hours

Voltage ---------- 7,500 (half wave)

Cell No.	pH of cell after electrolysis	Percentage of total gallic acid in cell	Percentage of total pantothenic acid in cell*
1	3.0	16.0	7.7
2	3.8	30.2	15.8
3	3.9	35.4	39.1
4	4.5	18.2	37.4
5	5.4	0.0	0
6	7.0	0.0	0
7	7.5	0.0	0
8	8.5	0.0	0

* With this run, no standards containing known amounts of rice bran extract were tested on yeast. Therefore no growth curve could be plotted in the usual way. These values were obtained by comparison of the various galvanometer readings among themselves.

TABLE V

Results of Electrolysis

Acid added ---------- Gallic acid

Amount ---------- 5 mg.

Time of electrolysis ------ 30 hours

Voltage ---------- 7,500 (half wave)

Cell No.	pH of cell after electrolysis	Percentage of total gallic acid in cell	Percentage of total pantothenic acid in cell		
			Test 1 (2 cc.)	Test 2 (1 cc.)	Test 3 (0.5 cc)
1	2.70	14.7	7.3	4.9	9.9
2	3.30	46.9	39.4	32.7	38.9
3	3.60	38.9	48.8	60.7	47.6
4	4.80	0.0	0	0	0
5	5.50	0.0	0	0	0
6	5.25	0.0	0	0	0
7	5.30	0.0	0	0	0
8	9.30	0.0	0	0	0

TABLE VI

Results of Electrolysis

Acid added ---------- Gallic acid

Amount ---------- 5 mg.

Time of electrolysis -------- 36 hours

Voltage ---------- 7,500 (half wave)

Cell No.	pH of cell after electrolysis	Percentage of total gallic acid in cell	Percentage of total pantothenic acid in cell	
			Test 1 (1 cc.)	Test 2 (0.5 cc.)
1	2.85	28.2	21.0	24.4
2	3.30	49.0	43.2	38.2
3	4.25	23.7	35.9	37.4
4	4.85	0.0	0	0
5	5.80	0.0	0	0
6	5.60	0.0	0	0
7	5.00	0.0	0	0
8	8.35	0.0	0	0

TABLE VII

Results of Electrolysis

Acid Added ---------- Pyrogallol

Amount ---------- 5 mg.

Time of electrolysis ------- 24 hours

Voltage ---------- 7,500 (half wave)

Cell No.	pH of cell after electrolysis	Percentage of total pyrogallol in cell	Percentage of total pantothenic acid in cell	
			Test 1 (1 cc.)	Test 2 (0.5 cc.)
1	2.85	7.8	12.2	10.4
2	3.70	46.5	41.1	41.7
3	4.20	23.6	46.8	49.1
4	5.10	6.4	0	0
5	4.80	6.5	0	0
6	5.00	4.9	0	0
7	6.50	(3.0)*	0	0
8	8.60	1.3	0	0

* The contents of this cell were accidentally lost. The amount was found by difference.

TABLE VIII

Results of Electrolysis

Acid added ---------- Salicylic acid

Amount ---------- 10 mg.

Time of electrolysis ------- 24 hours

Voltage ---------- 7,500 (half wave)

Cell No.	pH of cell after electrolysis	Percentage of total salicylic acid in cell *
1	3.25	64.5
2	3.40	27.2
3	3.85	8.2
4	4.75	0
5	5.15	0
6	5.45	0
7	6.50	0
8	8.20	0

* As salicylic acid is toxic to yeast, the amounts of pantothenic acid in the various cells could not be determined in this particular case. Deduction can be made only on the constant behavior of pantothenic acid in other electrolyses, similarly run.

TABLE IX

Results of Electrolysis

Acid added ---------- Caproic acid

Amount ---------- 25 mg.

Time of electrolysis ------ 24 hours

Voltage ---------- 7,500 (half wave)

Cell No.	pH of cell after electrolysis	Percentage of total caproic acid in cell*	Percentage of total pantothenic acid in cell	
			Test 1 (1 cc.)	Test 2 (0.5 cc.)
1	2.75	26.2	23.2	27.7
2	3.60	21.4	35.0	37.1
3	4.00	19.3	26.6	21.9
4	4.00	16.8	15.3	13.5
5	4.20	11.2	0	0
6	4.90	0	0	0
7	4.85	2.7	0	0
8	8.35	0	0	0

* See discussion of analytical method, page 8

TABLE X

Results of Electrolysis

Acid added ---------- Benzoic acid

Amount ---------- 25 mg.

Time of electrolysis ------- 24 hours

Voltage ----------7,500 (half wave)

Cell No.	pH of cell after electrolysis	Percentage of total benzoic acid in cell*	Percentage of total pantothenic acid in cell		
			Test 1 (2 cc.)	Test 2 (1 cc.)	Test 3 (0.5 cc)
1	2.65	23.9	13.7	15.9	16.4
2	3.50	18.6	19.4	16.7	21.0
3	3.65	18.6	21.3	22.4	22.0
4	3.85	16.6	26.6	26.9	19.9
5	5.20	10.7	20.1	18.2	20.7
6	5.10	4.5	0	0	0
7	5.30	4.2	0	0	0
8	8.80	3.0	0	0	0

* See discussion of analytical method, page 8.

TABLE XI

Ionization Constants[7]

α -hydroxy acids

1.	Lactic acid	$1.4 \cdot 10^{-4}$
2.	α hydroxy valeric acid	$2.6 \cdot 10^{-4}$
3.	α hydroxy butyric acid	$1.06 \cdot 10^{-4}$
4.	α hydroxy, α,β,β, trimethyl propionic acid	$1.14 \cdot 10^{-4}$

β -hydroxy acids

1.	β hydroxy butyric acid	$3.1 \cdot 10^{-5}$
2.	β hydroxy propionic acid	$3.5 \cdot 10^{-5}$

γ -hydroxy acids

1.	γ hydroxy butyric acid	$1.93 \cdot 10^{-5}$
2.	γ hydroxy valeric acid	$2.07 \cdot 10^{-5}$

Discussion of Results:

It may be seen from the first three electrolyses (Tables I,II,III) that the distribution of gallic acid is not affected by its concentration in the solution. This is as predicted by the theory. By analogy, it is concluded that the concentration of pantothenic acid in the extract does not determine the position in which it is found after electrolysis. This conclusion is also supported by experiment.[8]

From an examination of the data in tables II, IV, V, and VI, it may be observed that the length of time of electrolysis has the same effect on the distribution of both gallic and pantothenic acids; viz, both acids are removed to a region of lower pH with more extended electrolysis. This is in accord with the theory as set forth above, and not with the behavior of amphoteric substances.

From the electrolysis involving pyrogallol (Table VII), it may be seen that pyrogallol ($k = 10^{-8}$) is much weaker than pantothenic acid, as it is not completely removed from even the most basic cell of the apparatus.

Electrolysis of salicylic acid solution (Table VIII) showed that salicylic acid ($k = 1.0 \cdot 10^{-3}$) is much stronger than pantothenic acid, as it was completely removed from a cell of pH 4.75, after running only 24 hours. It was

nearly removed from a cell of pH 3.85, where pantothenic acid would be most concentrated under similar conditions.

Since in the cases of caproic acid and benzoic acid, the analytical methods were not satisfactory, it can only be remarked that the strength of pantothenic acid is of the same order of magnitude as the strengths of caproic acid ($k = 1.46 \cdot 10^{-5}$) and benzoic acid ($k = 6.64 \cdot 10^{-5}$).

In all the electrolyses involving gallic acid ($k = 3.9 \cdot 10^{-5}$), gallic acid was concentrated at a slightly lower pH than pantothenic acid. It is therefore concluded that pantothenic acid is slightly weaker than gallic acid. Its ionization constant is probably $1.5 - 3.5 \cdot 10^{-5}$.

Gallic acid was chosen for the determination of the effects of concentration and length of electrolysis on distribution, because in preliminary runs, its behavior was found to be very similar to that of pantothenic acid. A secondary consideration was the simplicity of the analytical procedure.

Pyrogallol and salicylic acid were chosen to determine roughly the effect on distribution, of rather widely divergent ionization constants.

Benzoic acid and caproic acid were used in an effort to narrow down the probable limits of the ionization constant of pantothenic acid. Unfortunately, these results

proved to be only rough checks.

From the fact that pantothenic acid appears to be slightly weaker than gallic acid ($k = 3.9 \cdot 10^{-5}$), it may be concluded from reference to table XI, in which various ionization constants are given, that it has no alpha hydroxyl group in its structure. It may have beta or more distant hydroxyl groups.

Summary:

1. By use of a method not previously applied, involving fractional electrolysis, the ionization constant of pantothenic acid is found to be somewhat less than that of gallic acid (k $3.9 \cdot 10^{-5}$).

2. As the ionization constants of all alpha hydroxy acids are approximately three or four times as large as that of gallic acid, it is concluded that it cannot have an alpha hydroxyl group in its structure. Beta or gamma hydroxyl groups would give it about the ionization constant which it appears to have.

Acknowledgement:

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