[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF OREGON STATE COLLEGE]

The Approximate Ionization Constant of Pantothenic Acid as Determined by Fractional Electrolysis

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A number of properties of pantothenic acid, the recently discovered growth determinant of universal biological occurrence, have been experimentally determined. Chemical evidence indicates that it is an aliphatic hydroxy acid of comparatively low molecular weight.

To throw further light on its character, and help in the problem of its chemical isolation, it was desirable to determine at least roughly its ionization constant. Since the acid has never been obtained in pure form and is detected both qualitatively and quantitatively only by its biological effect (on yeast growth) obviously none of the usual methods could be used to determine its acid strength.

The use of fractional electrolysis, as illustrated below, gives us a method whereby an approximate value is obtained.

We made use of the eight-compartment electrolysis apparatus utilized in previous studies[1] and for obtaining a suitable source of d. c. current one-half of a secondary of a 15,000 volt G. E. Luminous Tube transformer and a G. E. Kenetron tube FP-85, were used.

During the course of a number of electrolyses of twenty-four hours duration in which 50 mg. of a preparation from rice bran was electrolyzed, it became apparent that the physiologically potent constituent behaves in a constant manner. It is always removed completely from those cells which at the end of the electrolysis have a *P*H value of 4.5–4.75 or greater, and becomes most concentrated at a *P*H value of about 3.8. Its migration from the basic end and failure to migrate appreciably after it reaches a *P*H of about 3.8 is due to its weak acid character and the repression of its ionization in the stronger acid medium. This repression is independent of the concentration of the acid.[1]

The method we employed involved a study of the behavior of acids of known ionization constants under the same conditions of electrolysis. In these experiments the pantothenic acid was determined by methods worked out in this Laboratory with the modification that the blank medium contained in addition to the constituents previously used, traces of inositol, thallium, zinc, manganese, boron, copper, iodine, and iron. A discussion of these modifications will appear in another paper.

Gallic acid was determined colorimetrically by taking advantage of the color produced when the solution is made alkaline.[2] Pyrogallol was determined in a similar manner. Salicylic acid was determined by acidifying, extracting with ether, taking up with alkali, neutralizing and treating with ferric alum and comparing the color with standards.[3] Hydrogen ion determinations were made with a quinhydrone electrode.

In Table I are given the results of an electrolysis when 5 mg. of pyrogallol ($K_A = 10^{-8}$, approximately) was electrolyzed for twenty-four hours, *in the presence of* 50 mg. of the rice bran preparation. It is apparent that pyrogallic acid is much weaker than pantothenic acid because appreciable amounts were left in the cells with higher *P*H value.

TABLE I

FRACTIONAL ELECTROLYSIS (PYROGALLOL)

Cell no.	*P*H 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	7.81	12.2	10.4
2	3.70	46.5	41.1	41.7
3	4.20	23.6	46.8	49.1
4–8	4.80–8.60	6.4–1.3	0	0

Table II gives the results of an electrolysis of 10 mg. of salicylic acid ($K_A = 10^{-3}$) in the presence of the rice bran material. As salicylic acid is toxic to yeast the amounts of pantothenic acid in the various cells after this electrolysis could not be determined directly. However, it is possible to make deductions from the fact that in numerous other electrolyses its behavior was substantially the same. Salicylic acid is very appreciably stronger than pantothenic acid because under like conditions it migrates from a cell with *P*H 3.85 to the most acid cell (*P*H 3.25).

TABLE II

FRACTIONAL ELECTROLYSIS (SALICYLIC ACID)

Cell no.	*P*H 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
4–8	4.75–8.20	0

From the above experiments it is apparent that the ionization constant of pantothenic acid lies between that of pyrogallic acid ($K_A = 10^{-8}$) and salicylic acid ($K_A = 10^{-3}$).

We were fortunate in striking upon an acid which can be determined in small amounts[2] and which upon electrolysis behaves very much like pantothenic acid. Tables III and IV show the results obtained when 2.5 mg. and 10 mg., re-

(1) Williams, Lyman, Goodyear, Truesdail and Holaday, THIS JOURNAL, **55**, 2912 (1933).

(2) Allen, "Commercial Organic Analysis," 4th ed., 1910, Vol. III, p. 528.

(3) *Ibid.*, Vol. III, p. 484.

TABLE III

FRACTIONAL ELECTROLYSIS (GALLIC ACID)

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	2.75	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
5–8	5.25–9.0	0	0	0	0

TABLE IV

FRACTIONAL ELECTROLYSIS (GALLIC ACID)

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
5–8	4.35–7.50	0	0	0	0

spectively, of gallic acid ($K_A = 3.9 \times 10^{-5}$) were electrolyzed in the presence of the standard rice bran material. It will be noted that the migration of the gallic acid is parallel to that of the pantothenic acid except that in each case the gallic acid migrates appreciably farther toward the acid end, indicating that it is appreciably stronger as an acid. Results exactly similar to these were obtained in four different electrolyses when 5 mg. of gallic acid was electrolyzed in the presence of the rice bran material, for eighteen, twenty-four, thirty and thirty-six hours, respectively. In every one of these electrolyses as in the ones detailed in Tables III and IV, gallic acid migrated a little farther toward the acid end of the system.

The fact that the ionization constant of pantothenic acid appears to be somewhat lower than 3.9×10^{-5}, is of considerable significance when it is realized that all α-hydroxy acids which have been studied have ionization constants of 10^{-4} or higher.[4] Evidently pantothenic acid is not an α-hydroxy acid. The approximate value obtained does not preclude its having beta or gamma hydroxyl groups since beta (and gamma) hydroxy acids are actually somewhat weaker than gallic acid.

Summary

1. A new method involving fractional electrolysis has been applied to the approximate determination of the ionization constant of a physiologically potent substance (pantothenic acid).

2. The ionization constant of pantothenic acid is found to be approximately that of gallic acid (3.9×10^{-5}) although slightly lower.

3. From comparison with the ionization constants of acids of known structure, it is concluded that pantothenic acid cannot be an alpha hydroxy acid but may possess beta or gamma hydroxyl groups.

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(4) Scudder, "Conductivity and Ionization Constants of Organic Compounds," 1914.

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The Pinacol–Pinacolone Rearrangement. V. The Rearrangement of Unsymmetrical Aromatic Pinacols

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Recently[2] an investigation of the rearrangement of unsymmetrical pinacols was begun; three pinacols of the type RR(OH)CC(OH)R′R′, in which R and R′ are aryl groups, were subjected to rearrangement and the extent to which each of the two groups migrated was determined. We are now reporting the results obtained by rearrangement of seven new pinacols of this kind. In Table I are shown the percentage migration of the two groups in each pinacol; for comparison there is shown the migration of the same groups when situated in the symmetrical pinacol RR′-(OH)CC(OH)RR′.

It is apparent that the groups migrate differently when situated in the two types of pinacols. In a number of cases the order of migration is reversed; that is, the group that migrates to the greatest extent in the symmetrical molecule migrates least when situated in the unsymmetrical pinacol. There is no simple relationship between the two sets of values.[3] This is even more apparent from a comparison of the series

(1) Submitted in partial fulfilment of the requirements for the Ph.D. degree.

(2) Bachmann, THIS JOURNAL, **54**, 2112 (1932).

(3) For comparison with pinacols containing the biphenylene group see Bachmann and Sternberger, *ibid.*, **55**, 3819 (1933).