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Tannic Acid, U. S. P.

II. Relationship between Glucose and Tannic Acid

By Clifton E. Miller and L. Wait Rising†*

It was claimed by Freda (1) in 1854 that tannic acid yielded on acid hydrolysis three molecules of gallic acid and one molecule of glucose, the glucose being a part of the tannic acid molecule. As a result of the study of the action of yeasts on tannic acid by Biddle (2) it was concluded that the glucose present in tannic acid is a free component and therefore an impurity.

EXPERIMENTAL

We endeavored to demonstrate the veracity of these seemingly conflicting theories by proving that glucose is not only a component part of tannic acid but an impurity as well. In order to do this, one per cent solutions of tannic acid were prepared and tested immediately by means of the Molisch reagent. A broad, distinct, violet band was formed. These one per cent solutions were then diluted to a strength of 1:1000. Using the Molisch reagent the violet band became narrower and fainter as the dilution increased. A solution freed of gallic acid also gave a positive test.

We therefore concluded that a sugar was present in the tannic acid samples as an impurity in varying amounts. As this reaction did not give any indication of the kind or amount of sugar, our attention was directed toward the possibility of preparing and isolating some derivative of the sugar in order to determine the kind and amount. If the amount of sugar was found to increase on hydrolysis it would prove possibly that the sugar existed in a combined form.

Osazones are usually crystalline in nature and can be easily purified by crystallization. The osazone prepared from the sugar in tannic acid after recrystallization from alcohol and pyridine was found to melt at 208° C. This led us to believe that the sugar was glucose and the fact that the sugar gave a negative Seliwanoff reaction was considered further proof of this.

The next step was the development of a method to determine the amount of osazone formed without the use of the tedious and unreliable gravimetric method. The unreliability of this method may be attributed to the solubility of the osazone in the large excess of phenylhydrazine acetate that is present in the mixture.

Knecht's method (3) of osazone estimation by means of titanium trichloride was modified by using methylthionine chloride instead of crystal scarlet. The solutions required were: standard titanium chloride solution prepared by mixing 50 cc. of commercial 20 per cent titanium chloride with 50 cc. of hydrochloric acid, specific gravity 1.175, and diluting to one liter with freshly distilled water; standard glucose solution containing 10 mg. per cc.; fifteenth molar methylthionine chloride solution; saturated sodium tartrate solution; and twenty-five hundredths per cent phenylhydrazine in glacial acetic acid.

The titanium chloride solution was standardized both in terms of milligrams of glucose per cc. of solution which is hereafter called the glucose factor and cc. of methylthionine chloride solution. The latter standardization was made at a temperature of 70° C. in the presence of hydrochloric acid. It is advisable to determine the glucose factor for each lot of commercial titanium chloride for accurate results. The end-point of the titration is the formation of a permanent blue color, which is advantageously seen by directing a beam of light from beneath the flask.

For the hydrolysis, a 20 per cent solution of each sample in approximately tenth-normal sulfuric acid was heated continuously on a steam bath. In order

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to determine the amount of glucose formed as a result of the acid hydrolysis, samples of each solution were taken for analysis prior to heating. During the heating procedure samples were withdrawn at the end of each five-hour period, cooled to room temperature and the glucose determined in the following manner.

Two cc., representing 40 mg. of tannic acid, of the hydrolysis mixture, two cc. of saturated sodium tartrate solution and two cc. of the phenylhydrazine solution were placed in a test-tube and heated in a boiling water bath for ten minutes. The hot solution was transferred to a small flat-bottom flask and a known excess of titanium chloride solution added. The contents of the flask were boiled over a free flame for two minutes. The mixture in the flask which had a chocolate color was acidified with seven and a half cc. of hydrochloric acid, specific gravity 1.175, and the hot solution titrated with methylthionine chloride to determine the excess titanium chloride. Having found the amount of titanium chloride used, the glucose factor was multiplied by the amount of titanium chloride to obtain the amount of glucose.

We were able to calculate the quantity of glucose formed during the hydrolysis by subtracting the amount found previous to the heating from that found at the completion of the hydrolysis. Constant results at the end of two five-hour periods determined the end-point of hydrolysis. The heating was continued beyond this point on several samples and it was found that there was no change in the results. The amount of sugar is designated in terms of milligrams per 40 mg. of tannic acid in Table I, and represents the total free glucose at each five-hour interval.

Table I.—Total Free Glucose at Each Five-Hour Period

Hours of Heating	Sample 1	2	3	4	5	6	7	8	9	10	11	12
0	5.15	2.97	3.31	2.87	2.28	4.65	3.06	3.55	2.33	2.62	2.81	2.60
5	4.51	2.77	4.75	5.05	3.96	5.35	2.89	2.40	2.71	2.96	2.57	3.30
10	4.35	3.86	3.26	5.35	3.66	6.53	3.26	3.87	2.89	2.89	2.64	3.01
15	6.10	3.36	4.31	5.25	3.46	5.25	3.31	3.50	3.62	2.52	3.01	3.87
20	5.84	4.16	4.73	5.38	4.31	5.14	3.38	3.87	3.38	2.89	3.13	3.74
25	6.10	3.17	5.09	4.45	3.07	4.95	3.84	4.24	3.62	3.87	3.62	3.62
30	5.19	3.36	3.75	5.44	3.46	4.54	4.65	3.85	3.74	3.99	3.74	3.74
35	5.74	4.36	5.22	5.80	3.82	6.63	4.48	4.36	3.71	3.74	3.87	3.71
40	6.34	3.06	4.12	4.85	4.45	4.95	4.65	3.87	3.92	3.74	4.11	4.68
45	5.94	4.95	5.34	5.28	4.12	5.74	5.34	4.36	4.25		5.34	4.71
50	5.74	4.65	5.07	4.85	4.26	5.74	5.09	5.09	4.36		4.48	4.82
55	5.94	4.35	6.19	6.23	3.96		5.95	5.09	4.94		5.21	4.71
60	6.10	4.35	3.95	5.84	4.26		5.95		4.65		5.58	5.09
65	6.83		4.85	6.15	4.61				4.72		5.29	4.11
70	6.34		5.34	6.47	6.04				5.21		5.46	5.44
75	7.92		7.17	7.03	6.04				5.46		5.70	5.56
80	6.63		5.83	6.63					5.46		5.28	6.44
85	6.75		5.83	6.53							5.95	5.95
90	8.51			8.01							5.95	5.70
95	7.62			7.33								5.58
100	8.21			7.33								6.32
105	8.21											6.05
110												6.05

Table I shows the amount of sugar formed as a result of the hydrolysis and the amount of sugar present as an impurity.

Table II.—Sugar Formed by Hydrolysis and as an Impurity

	Sample 1	2	3	4	5	6	7	8	9	10	11	12
Total sugar, %	20.40	10.80	14.50	19.20	15.00	14.20	14.30	12.70	13.60	9.30	14.90	15.10
Sugar as impurity, %	12.80	7.10	8.20	7.50	5.60	11.60	7.60	8.80	5.80	6.30	7.00	6.70
Sugar formed, %	7.60	3.70	6.30	11.70	9.40	2.60	6.70	3.90	7.80	3.00	7.90	8.40

A possible explanation of the vacillation in the sugar formation is that glucose and gallic acid in the presence of an acid may unite to form an unstable compound which will break down to give sudden maximum concentrations of glucose. It may also show that in some samples there is relatively more gallic acid present as an impurity.

CONCLUSIONS

1. The acid hydrolysis of Tannic Acid, U. S. P. has been studied quantitatively with respect to its glucose content.

2. A quantitative method for the determination of glucose in Tannic Acid has been developed.

3. It is evident that the glucose is present in variable amounts as an impurity and for this reason different samples of the same weight of Tannic Acid would not necessarily yield the same amount of glucose on hydrolysis.

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Tannic Acid, U. S. P.

III. Hydrogen-Ion Studies of Tannic Acid Solutions

By Clifton E. Miller and L. Wait Rising†*

It is well known that freshly prepared solutions of tannic acid in water, irrespective of concentration, show a marked change in color, formation of bacterial growth, formation of an odor and, in some cases, deposition of a residue when kept over a prolonged period of time. These changes appear to be the result of decomposition. Engels (1) and Fantus (2) introduced various stabilizing agents in an attempt to prevent these changes. Therefore it was desired to measure the degree of decomposition of the stabilized and unstabilized solutions if possible. One way to do this on a comparative basis is to measure the resultant changes in hydrogen-ion concentration.

Surprisingly little work has been done on the hydrogen-ion value of tannic acid solutions. Johnson (3) in his survey of hydrogen-ion studies makes no mention of tannic acid-p_H relationship; Glasstone (4) proposed the limits of p_H in which it was possible to obtain a violet color by the addition of ferrous tartrate solution to solutions of tannic acid. This was another reason why the study seemed worth while.

EXPERIMENTAL

As tannic acid is water-soluble and does ionize, it seemed of interest to make a relative study of the hydrogen-ion concentration of freshly prepared solutions and stabilized solutions of tannic acid over varying intervals of time.

Therefore freshly prepared one per cent aqueous solutions were tested at varying intervals for a period of 96 hours. The hydrogen-ion value was determined using a saturated calomel-quinhydron, electrode. All readings were made at 250° C.

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Table I.—p_H Values of Freshly Prepared Tannic Acid Solutions

Sample	Hours 0	24	72	96
1	2.5	2.5	2.6	2.8
2	2.6	3.1	2.9	3.0
3	2.6	2.8	3.1	3.2
4	2.4	2.6	2.8	3.1
5	2.8	2.8	3.0	3.4
6	2.7	2.7	3.3	3.2
7	2.8	2.7	3.2	3.1
8	3.8	3.4	3.6	3.5
9	3.6	2.9	3.6	3.0
10	3.6	2.9	3.2	3.2
11	3.4	3.2	3.1	3.4
12	3.2	2.9	3.0	3.4

It is quite evident that aqueous solutions of tannic acid are unstable on standing and their internal constitution is not only constantly changing but generally toward the alkaline side.

As mentioned earlier, Fantus brought forth a formula for the stabilized solutions of tannic acid in which he proposed the use of stabilizers such as salicylic acid and sodium chloride. Solutions were prepared using the formula suggested by Fantus which is given herewith.

Potassium chloride	0.42 Gm.
Calcium chloride	0.84 Gm.
Salicylic acid	1.00 Gm.
Sodium chloride	10.50 Gm.
Tannic acid	100.00 Gm.
Distilled water *q. s.*	1000.00 Gm.

The p_H of the solutions was determined upon preparation and at two-month intervals over a period of 12 months. There was a general darkening of the solutions as time progressed. Generally the acidity decreased in proportion to the increase in time. A sediment was noted in the bottoms of the containers which were kept under ordinary laboratory conditions.

Table II.—p_H Values Using Fantus Formula at Two-Month Intervals

Sample	Months 0	2	4	8	8	10	Av.
1	3.0	2.7	2.4	2.5	2.6	2.6	2.6
2	2.9	3.3	2.8	2.9	3.2	3.4	3.1
3	3.1	4.3	4.0	4.4	4.2	4.1	4.0
4	2.8	4.0	3.2	3.6	3.2	3.2	3.3
5	3.1	3.9	3.8	2.2	4.0	3.9	3.5
6	2.7	4.4	3.7	4.0	3.8	4.0	3.8
7	2.5	4.0	3.6	4.2	3.5	3.9	3.6
9	2.9	4.2	3.6	4.1	3.6	4.1	3.7
10	2.9	4.3	3.7	4.2	4.2	3.7	3.9
11	2.9	4.5	3.6	3.7	3.8	3.8	3.7
12	2.9	4.2	3.7	3.9	3.5	3.7	3.7

Since Sample No. 8 presented difficulties in getting a clear solution using the formula of Fantus no results are given.

Solutions were also prepared using Engels' formula, which is given herewith.

Alkyl or aryl *p*-hydroxy benzoate	0.1000 Gm.
Sodium bisulfite	2.5000 Gm.
Tannic acid	50.0000 Gm.
Distilled water *q. s.*	1000.0000 Gm.