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An Improvement of the Method for the Determination of Galactan.

By

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The presence of galactan (or substances containing a galactose group in the molecule) in natural products has been shown, in recent years, by numerous investigators.

Quantitative determination of galactan has therefore been deemed desirable and the following method¹⁾, the underlying principle of which had been worked out by Tollens and his associates,²⁾ was provisionally adopted by the association of the official agricultural chemists, U. S. A., for its determination in foods and feeding stuffs.

“Extract a convenient quantity of the substance, representing from 2.5 to 3.0 grams of the dry material, on a hardened filter with five successive portions of 10 cc. of ether, place the extracted residue in a beaker about 5.5 cm. in diameter and 7 cm. deep, together with 60 cc. of nitric acid of 1.15 specific gravity, and evaporate the solution to exactly one-third its volume in a water bath at a temperature of 94° to 96°C. After standing twenty-four hours, add 10 cc. of water to the precipitate, and allow it to stand

1) U. S. Department of Agriculture, Bureau of Chemistry-Bulletin No. 107 (Revised).
Official and Provisional Methods of Analysis, P. 55.

2) B. Tollens- Kurzes Handbuch der Kohlenhydrate, II, Breslau, 1895, P. 52.

another twenty-four hours. The mucic acid has in the meantime crystallized, but it is mixed with considerable material only partially oxidized by the nitric acid. Filter the solution therefore through filter paper, wash with 30 cc. of water to remove as much of the nitric acid as possible, and replace the filter and contents in the beaker. Add 30 cc. of ammonium carbonate solution, consisting of 1 part ammonium carbonate, 19 parts water, and 1 part strong ammonium hydroxid, and heat the mixture on a water bath, at 80°C., for fifteen minutes, with constant stirring. The ammonium carbonate takes up the mucic acid, forming the soluble mucate of ammonia. Then wash the filter paper and contents several times with hot water by decantation, passing the washings through a filter paper, to which finally transfer the material and thoroughly wash. Evaporate the filtrate to dryness over a water bath, avoiding unnecessary heating which causes decomposition, add 5 cc. of nitric acid of 1.15 specific gravity, thoroughly stir the mixture and allow to stand for thirty minutes. The nitric acid decomposes the ammonium mucate, precipitating the mucic acid; collect this on a tared filter or gooch, wash with from 10 to 15 cc. of water, then with 60 cc. of alcohol, and a number of times with ether, dry at the temperature of boiling water for three hours, and weigh. Multiply mucic acid by 1.33 which gives galactose, and multiply this product by 0.9 which gives galactan."

The results obtained by the method above described are far from being accurate, at least in the case of natural products, according to the experiences in our laboratory. The chief reasons for the inaccuracy seem to us to be the following.

1) The presence of partially decomposed organic material in the solution appears to hinder greatly the crystallization of the mucic acid formed by oxidation with nitric acid. The fact is well illustrated by the experimental results which follow.

Table I.

Sample.	Mucic acid obtained.
Natural stuff ¹⁾ alone (3 g)	g 0.1214

1) Cotyledon of soy-bean.

Lactose alone (1 g)	0.3446
Total	0.4660
Natural stuff (3 g) and Lactose (1 g) together	0.2077
Loss	0.2583

It is thus seen that in the case of natural stuff and lactose in combination a considerable portion of the mucic acid was prevented from crystallization and lost in the filtrate.

2) In extracting the mucic acid with ammonium carbonate solution to free it from the admixed impurities, the partially oxidized organic material is dissolved, more or less, by the reagent used. The dissolved organic material is again precipitated together with the mucic acid, upon evaporation and subsequent addition of nitric acid. It is perhaps needless to remind those who have had the experience in this line of work that the final mucic acid obtained very often has a dirty appearance, clearly indicating the presence of some other organic matter admixed.

Attempts have therefore been made by us to improve the method and avoid the inaccuracies already pointed out.

By repeated trials with natural stuffs it has been found that the partially oxidized organic material could be separated completely from the solution containing mucic acid not yet crystallized, upon filtering immediately after evaporation with nitric acid to exactly one-third its volume. To determine whether or not the amount of mucic acid yielding substance affects the time required for crystallization of the acid under the given circumstances, the following experiments were undertaken with lactose¹⁾.

Table II.

Lactose.	Time required for crystallization of mucic acid.
g	
3.00	Immediately after evaporation.
1.50	” ” ”
1.00	” ” ”
0.50	On the following day.

1) The sugar used contained 5.035 % water.

0.30	}	On the following day.
		Not crystallized even after standing 48 hours.
0.10		When evaporated down to one-fifth its original volume the crystals began to appear after several days.
0.05		

It will be seen from the table that down to one gram of lactose the mucic acid is precipitated immediately after evaporation. The case may or may not be the same, if organic matter is present in a considerable quantity in the solution. To test how far the presence of organic substance affects the time of crystallization of mucic acid the following experiment was made.

Table III.

Sample.	Time required for crystallization of mucic acid.
Natural stuff ¹⁾ 3 g + Lactose 3.0 g	Immediately after evaporation.
" " " + " 1.5 g	" " "
" " " + " 1.0 g	Several hours after evaporation.

In 3 grams of the natural stuff and 1 gram of lactose used in the experiment there was contained 16.38% of equivalent galactan on basis of dry matter.

From the results, it can be inferred that in the determination of galactan in natural products containing galactan up to the above limit, the immediate filtration after evaporation will not cause any loss of mucic acid.

The Proposed Method.

On the basis of the above experiments the following modification in the procedure of the method is here proposed and recommended.

Take a given quantity of the substance to be examined, extract with ether and place the residue in a beaker, together with nitric acid and evaporate the solution in a water bath, exactly in the same manner as recommended in the "provisional method" already referred to. Filter hot immediately after evaporation and wash well with hot water. The filtrate is again evapo-

1) Cotyledon of soy-bean.

rated down to one-third volume of the original solution. After allowing to stand twenty four hours; add 10 cc. of water and allow it to stand another twenty four hours or longer if necessary. In the mean time the mucic acid will be crystallized. Collect the mucic acid on a tared filter or gooch, wash with cold water, then with alcohol, and finally with ether, dry at the temperature of boiling water and weigh.

In this process, the use of ammonium carbonate solution is entirely dispensed with, as it has been found by actual experiments that the results with or without the purification process are in perfect accord. The results of the experiments follow.

Table IV.

Sample.	Method.	Mucic acid obtained.
Natural stuff ¹⁾	Not purified.	0.1557 ^g
	Purified with ammonium carbonate solution & nitric acid.	0.1555
Natural stuff ²⁾	Not purified.	0.0859
	Purified with ammonium carbonate solution & nitric acid.	0.0863

The amount of the sample to be taken for analysis must be determined according to its galactan content. If its content is below 16% on dry matter basis, 3 grams can be taken but if more galactan is contained, then a correspondingly smaller amount of the substance must be used, as otherwise the mucic acid is liable to crystallize out before the filtration. On the other hand, if the amount of galactan is insignificant, and below 0.8 %, the filtrate should be evaporated down into one-fifth its original volume, and allowed to stand several days if necessary; otherwise the mucic acid may fail to crystallize even after long standing and it may lead to an erroneous conclusion.

Comparison of the Results.

The tables given below show the results obtained by the provisional as

1) Cotyledon of soy-bean

2) Cotyledon of 2 weeks seedling.

well as proposed method.

Table V.

Sample used.	Method.	Mucic acid obtained.	Mucic acid obtained from filtrate on further evaporation.	Total.
		g	g	g
Natural stuff ¹⁾ 3 g	Provisional	0.1214	0.0448	0.1662
	Proposed	0.1557	—	0.1557
Natural stuff ²⁾ 3 g	Provisional	0.0741	0.0258	0.0999
	Proposed	0.0859	—	0.0859
Natural stuff ³⁾ 3 g	Provisional	0.0722	0.0276	0.0998
	Proposed	0.0804	—	0.0804
Natural stuff ⁴⁾ 3 g	Provisional	0.0398	0.0177	0.0575
	Proposed	0.0460	—	0.0460
Natural stuff ⁵⁾ 3 g	Provisional	0.0815	0.0347	0.1152
	Proposed	0.0587	—	0.0587

Table VI.

Sample used.	Method.	Mucic acid obtained.	Mucic acid obtained from filtrate on further evaporation.	Total.
		g	g	g
Natural stuff ¹⁾ 3 g + Lactose 0.3 g	Provisional	0.2344	0.0558	0.2902
	Proposed	0.2528	—	0.2528
Natural stuff ²⁾ 3 g + Lactose 0.3 g	Provisional	0.1783	0.0206	0.1989
	Proposed	0.1787	—	0.1787
Natural stuff ³⁾ 3 g + Lactose 0.3 g	Provisional	0.1508	0.0277	0.1785
	Proposed	0.1432	—	0.1432

1) Cotyledon of soy-bean.

2) Cotyledon of 2 weeks seedling.

3) 2 weeks seedling without cotyledon.

4) Cotyledon of 4 weeks seedling.

5) 4 weeks seedling without cotyledon.

Lactose for the experiments contained 5.035% of water.

Table VII.

Method.	Sample.	Total mucic acid obtained from		Diffe- rence.	Mucic acid obtained from 0.3 g lactose.
		3 g sample + 0.3 g lactose.	3 g sample alone.		
		g	g	g	g
Provisional method	Natural stuff ¹⁾	0.2902	0.1626	0.1240	0.0933
	Natural stuff ²⁾	0.1989	0.0999	0.0990	"
	Natural stuff ³⁾	0.1785	0.0575	0.1210	"
Proposed method	Natural stuff ¹⁾	0.2528	0.1557	0.0971	"
	Natural stuff ²⁾	0.1787	0.0859	0.0928	"
	Natural stuff ³⁾	0.1432	0.0460	0.0972	"

It will be seen from the figures that the provisional method gives mucic acid usually less in amount than the proposed method. In the former method, when the filtrate of the solution after separating the mucic acid and partially oxidized material, is again concentrated, a not inconsiderable amount of the mucic acid is recovered. In the ordinary operation of the method this amount is lost entirely in the filtrate. The sum of two crops of the mucic acid exceeds always that obtained by the proposed method. This difference is due, in our judgment, to the admixture of the organic substance dissolved out by ammonium carbonate solution. The loss of mucic acid in the filtrate is compensated to some extent by the dissolved organic materials. But the loss in the filtrate is usually too great to be counterbalanced in this way. It is thus seen that the results obtained by the provisional method are not accurate and have little value beyond the qualitative significance for the presence of galactan or other mucic acid yielding substances.

1) Cotyledon of soy-bean.

2) Cotyledon of 2 weeks seedling.

3) Cotyledon of 4 weeks seedling.

Factor for the Calculation of Galactan.

To calculate the amount of galactose from the mucic acid obtained, the factor 1.33 is commonly used. Multiplying the galactose by 0.9 gives the quantity of galactan. The factor 1.33 is deduced from the fact that 100 parts of galactose yield about 75 parts of mucic acid by oxidation with nitric acid in the manner specified elsewhere. The yield of mucic acid is not, however, constant but varies with the amount of substance taken and the method of operation, as is pointed out by Tollens¹⁾ and Creydt²⁾. We have verified the same by oxidizing the different amounts of lactose with 60 cc. nitric acid, sp. gr. 1.15. The results are shown in the following table.

Table VIII.

Lactose ³⁾ taken.	Mucic acid obtained.	% of mucic acid. (water free basis)	% × 2.
g	g		
3.00	1.0951	38.44	76.88
1.00	0.3446	36.29	72.58
0.30	0.0933	32.75	65.50
0.10	0.0194	20.43	40.86

It will be seen from the table that the greater the amount of lactose taken, the higher is the percentage yield of mucic acid. It is consequently reasonable in the calculation of galactose to use different factors according to the amounts of mucic acid produced. The use of the factor 1.33, it should be understood, is for the sake of brevity and convenience.

1) B. Tollens - Kurzes Handbuch der Kohlenhydrate, I, Breslau, 1898, P. 317.

2) Lippmann - Chemie der Zuckerarten, I, Braunschweig, 1904, P. 1652.

3) Lactose for the experiment contained 5.035 % of water.

Summary

An improvement of the method for the determination of galactan in foods and feeding stuffs has been worked out and described. The proposed method is simpler in operation and gives far better results than the "provisional method" now in common use.

In conclusion, the author wishes to express his hearty thanks to Prof. Dr. K. Oshima for his kind advices in the course of present investigation. The author is also under obligation to Mr. H. Takahashi for his help in analytical work.