CLXII. THE ANALYSIS OF CARBOHYDRATES OF THE CELL WALL OF PLANTS

IV. DETERMINATION OF METHYLPENTOSE AS METHYLFURFURALDEHYDE: METHODS OF DISTILLATION AND PRECIPITATION

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ALTHOUGH Votocek [1897] was probably the first investigator to describe a method of determination of methylfurfuraldehyde by precipitation with phloroglucinol in hydrochloric acid solution, it was left to Ellett & Tollens [1905], following the work of Kröber [1901] on pentoses, to apply the method to the methylpentoses. By the usual distillation procedure with 12% hydrochloric acid and precipitation with phloroglucinol they were able to construct tables showing the relationship between rhamnose and methylfurfuraldehydephloroglucide. In addition, they sought to apply the method to mixtures of arabinose and rhamnose, separating the precipitated phloroglucides by extraction with boiling alcohol in which the furfuraldehyde precipitate was supposedly insoluble. The accuracy of the procedure was of no very high order, a fact which Ellett & Tollens admitted; but nevertheless, the method as originally proposed is in wide use at the present time, having been adopted, for example, by the Association of Official Agricultural Chemists.

The determination naturally falls into two parts: (a) the distillation; (b) the determination of methylfurfuraldehyde. With regard to the former, it has been shown in a previous communication [Marshall & Norris, 1937] that the yield of methylfurfuraldehyde depends on the original sugar itself and also on such factors as oxidation and the action of the acid, which are inherent in the distillation procedure. The investigations to be described in this communication are divided into three sections: (a) a review of the distillation procedure with suggestions for that which is best suited to the methylpentoses; (b) a review of precipitation and titrimetric methods for determining methylfurfuraldehyde; and (c) the isolation and identification of a substance, derived by distillation from rhamnose, and shown to be acetone, which is a source of error in some methods of determination.

EXPERIMENTAL

A. *Distillation*

Changes in the acid concentration during the distillation are unquestionably the major source of decomposition of methylfurfuraldehyde when rhamnose itself is under treatment. Hence it appears probable that the most suitable distillation procedure to adopt would be such that no changes occurred in the concentration of the acid.

In the method of Ellett & Tollens, which employs 12 % hydrochloric acid the concentration of acid is far from constant during the period of distillation but since the existing tables and formulae are based on this distillation method, it was deemed advisable to test this carefully before seeking others.

Method of Ellett & Tollens. The method employed by us for distillation and subsequent precipitation with phloroglucinol was similar to that originally devised by Ellett & Tollens for methylpentoses, but was very stringently standardized, and has been described in detail in previous communications in this series, being closely similar to that used by Kröber for pentose determinations.

Determinations on samples of rhamnose of well-defined purity were carried out by this method, the results being compared with those obtained by Ellett & Tollens as set out in their tables reproduced by van der Haar [1920].

The following table is representative of a large number of results obtained, and it is evident that there is considerable lack of concordancy between duplicates, and that in every case our yields of phloroglucide were lower than those recorded by Ellett & Tollens. The yield of phloroglucide is smaller and the discrepancy with the earlier workers greater for the smaller quantities of rhamnose.

Table I

Wt. of rhamnose g.	Wt. of phloroglucide		Yield calc. on rhamnose	
	Found g.	Ellett & Tollens g.	Found %	Ellett & Tollens %
0.0508	0.0138	0.0255	27.16	50.19
	0.0144		32.28	
	0.0164		28.34	
	0.0145		28.15	
0.0606	0.0251	0.0318	41.42	52.47
0.0615	0.0285	0.0326	46.34	53.01
0.0995	0.0556	0.0580	55.89	58.29
	0.0557		55.89	
	0.0462		46.45	
	0.0552		55.47	
	0.0480		48.23	
	0.0511		51.36	

The standard error of experiment was high, being of the order of $\pm 8\%$. That the method of distillation was essentially responsible for the discrepancies observed was substantiated by distilling a large quantity of rhamnose in a similar manner and then removing aliquots of the distillate for precipitation with phloroglucinol and thiobarbituric acid, with the following typical results.

Table II

Wt. of ppt. with phloroglucinol g.	Wt. of ppt. with thiobarbituric acid g.
0.0419	0.0528
0.0418	0.0531
0.0428	0.0533
0.0428	0.0531

The standard errors here are much smaller and of the order to be expected, being $\pm 1.2\%$ for phloroglucinol and $\pm 0.7\%$ for thiobarbituric acid.

Steam-distillation. The distillation procedure of Ellett & Tollens having proved unsatisfactory, we then sought to determine whether the steam-distillation

method advocated by Pervier & Gortner [1923] could be applied successfully to the problem. Here again it must be stressed that the acid concentration does not remain constant during distillation, but in this case the acid in the flask becomes progressively weaker as distillation proceeds. Theoretically, then, higher and possibly more concordant yields of methylfurfuraldehyde should be obtained.

On account of the large volumes of distillate that were obtained by this method, the methylfurfuraldehyde was determined titrimetrically by the method of Powell & Whittaker [1924]. By reference to Table III it will be observed that the discrepancies between determinations were even greater than in the previous method, and the percentage recovery of methylfurfuraldehyde was actually lower than that obtained by Ellett's distillation method.

Table III

Wt. of rhamnose g.	Methylfurfuraldehyde		
	Theory g.	Found g.	Recovery %
0.1993	0.1205	0.0600	49.84
		0.0595	49.38
		0.0587	48.73
		0.0565	46.88
0.1018	0.0615	0.0249	40.33
		0.0282	45.89
		0.0321	52.19

Distillation procedure of Kullgren & Tyden [1929]. Finally, we essayed the distillation method suggested by Kullgren & Tyden. These authors recommended distillation of the pentose or methylpentose with 13.15% hydrochloric acid saturated with salt, the presence of the latter maintaining the acid concentration at 13.15% throughout the distillation owing to a depression of the ionization [Oman, 1924].

100 ml. of 13.15% hydrochloric acid containing a known weight of the sugar in solution are placed in the distillation flask, and the acid saturated by the addition of 20 g. sodium chloride. Distillation is continued at the rate of 25 ml. every 10 min., the liquid in the flask being replenished by repeated addition of fresh acid until distillation is complete.

Preliminary experiments had indicated that 20 g. of salt were usually insufficient to maintain the solution constantly saturated, with the result that a correspondingly detrimental effect on the yields of precipitate obtained was observed. Accordingly distillations were performed with varying quantities of salt (previously dried at 100°), and it was found that in proportion to the amount of salt added, the yields of methylfurfuraldehyde increased, as also the con-

Table IV

Wt. of salt g.	Wt. of ppt. with thiobarbituric acid g.
18	0.0745
	0.0690
20	0.0740
	0.0716
22	0.0763
	0.0766
24	0.0768
	0.0760

cordance between duplicate determinations. 22 g. of salt were just sufficient to ensure that the solution remained saturated without having a large excess present throughout the distillation, and hence this amount was employed in all subsequent determinations. The effect of increasing proportions of salt is shown in Table IV.

In the past, doubt has occasionally been expressed, particularly where titrimetric methods were used, as to whether all the methylfurfuraldehyde from rhamnose passed over with the first 350 ml. of distillate. We have found, however (Table V), that a distillation period of 2 hr. 20 min. suffices for complete distillation of the methylfurfuraldehyde, since a longer time of distillation shows no appreciable difference in the weight of precipitate obtained with thiobarbituric acid for example.

Table V

0.0960 g. rhamnose used in each case

Time of distillation min.	Wt. of ppt. with thiobarbituric acid g.
140	0.0732
140	0.0707
280	0.0732
280	0.0704

Using the technique outlined, the distillation procedure was then applied to the determination as phloroglucide of methylfurfuraldehyde from rhamnose. Good agreement was recorded between duplicate determinations, and in all cases the yields of phloroglucide were considerably higher than those given in the tables of Ellett & Tollens from the corresponding quantities of rhamnose.

Table VI

Wt. of rhamnose g.	Wt. of phloroglucide		Phloroglucide calc. on rhamnose	
	Found g.	Ellett & Tollens g.	Found %	Ellett & Tollens %
0.0754	0.0473	0.0415	62.73	55.03
	0.0468		62.05	
	0.0483		62.04	
	0.0471		62.46	
	0.0482		63.92	
	0.0481		63.78	
0.0997	0.0665	0.0581	66.69	58.27
	0.0665		66.69	
	0.0662		66.39	
	0.0665		66.69	
0.1502	0.1048	0.0951	69.76	63.31
	0.1058		70.46	
	0.1077		71.71	
	0.1059		70.50	

Thus the distillation has been exposed as the greatest weakness in the method of determination employed by Ellett & Tollens, but satisfactory results may be obtained if the distillation procedure of Kullgren & Tyden be adopted in the slightly modified form indicated.

B. Determination of methylfurfuraldehyde in hydrochloric acid solution

(1) *Precipitation methods.* The behaviour of the following reagents towards methylfurfuraldehyde and their use for determining methylfurfuraldehyde produced from rhamnose by distillation has been investigated: phloroglucinol,

barbituric acid, thiobarbituric acid, 2:4-dinitrophenylhydrazine, diphenylthio-barbituric acid.

Since in most cases the optimum conditions of precipitation with these reagents have not hitherto been determined, and since standardization in this phase of the determination is as essential as in the distillation, we give below details of procedure which we have found most satisfactory in each case.

Phloroglucinol. 50 ml. of phloroglucinol solution were added to 350 ml. of a standard methylfurfuraldehyde-hydrochloric acid solution. After standing 16 hr. the precipitates were filtered off through IG 4. sintered-glass crucibles, washed with 150 ml. water and dried at 100° for 4 hr.

There is little evidence that a definite compound of methylfurfuraldehyde and phloroglucinol is formed, and hence the relationship between the former and its phloroglucide is a purely empirical one. Such relationships have been determined mathematically on lines similar to those described in a previous communication [Angell *et al.* 1936]. Complete data are given in the following communication in this series but for the present it will suffice merely to indicate the derived relationship:

$$M = 0.5P + 0.0053 \pm 0.0017,$$

where M = g. methylfurfuraldehyde, P = g. phloroglucide corresponding.

Barbituric acid. A quantity of barbituric acid in 13.15% hydrochloric acid solution equivalent to eight times the weight of methylfurfuraldehyde anticipated was added and the final volume adjusted to 400 ml. by further addition of hydrochloric acid. After standing 40 hr., the precipitate was filtered through an alundum crucible (medium grade) and washed with 200 ml. of distilled water, dried for 4 hr. at 100°, cooled and weighed.

It was found that barbituric acid was not a very satisfactory precipitant in actual tests on rhamnose etc., and for this reason the direct relation between methylfurfuraldehyde and the barbituric acid precipitate was not determined. When required the formula of Fromherz [1907] was employed:

$$M = 1/2 [B + (n \times 0.000023)],$$

where M = g. methylfurfuraldehyde, B = g. barbituric acid precipitate, n = ml. solution.

Thiobarbituric acid. This reagent was prepared in the laboratory by the synthesis of Gabriel & Colman [1904].

We have found that it is advisable to use a freshly prepared solution of the reagent in 13.15% hydrochloric acid, which, after cooling, has been filtered to remove traces of insoluble substances. The proportion of thiobarbituric acid to methylfurfuraldehyde should be not less than 3:1. After addition of the reagent, the final volume is made up to 500 ml. The solution is allowed to stand 20–24 hr. after precipitation and then filtered through alundum crucibles (medium grade), washed with 150 ml. water and dried at 100° for 4 hr.

In this case the equation correlating methylfurfuraldehyde and its precipitate with thiobarbituric acid was found to be:

$$M = 0.4605T + 0.0008 \pm 0.0010,$$

where M = g. methylfurfuraldehyde, T = g. thiobarbituric acid precipitate.

2:4-Dinitrophenylhydrazine. An excess of a 0.5% solution of the reagent in 13.15% acid was employed. The weight of reagent used must be at least ten times the weight of methylfurfuraldehyde to be precipitated. After standing 24 hr. the precipitate is filtered through an alundum crucible, washed with 150 ml. water, dried at 100° for 2 hr., cooled and weighed.

The condensation product so obtained is quite insoluble and possesses a definite composition. Hence in this case methylfurfuraldehyde was calculated as required by multiplying by the factor 110/290.

Diphenylthiobarbituric acid. The reagent was prepared according to the procedure outlined by Tischtschenko & Koschkin [1934] and used according to their directions for the determination of furfuraldehyde. Although we found that this reagent does precipitate methylfurfuraldehyde, the results were entirely unsatisfactory and have not been included in this paper. It was found that excess of the reagent itself was precipitated together with the methylfurfuraldehyde condensation product. Attempts to remove the reagent from the filtered product by washing with larger or smaller quantities of 4% ammonium acetate, whether hot or cold, also proved unsatisfactory, the condensation products themselves being leached out together with excess of the reagent.

(2) *Titrimetric methods.* *Method of Powell & Whittaker [1924].* Perusal of the literature reveals that some doubt exists as to the precise amount of bromine absorbed by a given quantity of methylfurfuraldehyde. Preliminary work with methylfurfuraldehyde showed that the bromine absorbed depended upon the temperature, the time of standing and the excess bromine present, as the results in the following table testify.

Table VII

0.0464 g. methylfurfuraldehyde used in each case

Temperature °C.	Time of standing hr.	N/10 bromate		Atoms bromine per mol. methylfur- furaldehyde
		Added ml.	Absorbed ml.	
18	1	25	19.01	4.508
18	3	25	19.97	4.736
18	6	25	21.58	5.117
18	1	50	19.20	4.553
18	3	50	20.10	4.767
18	6	50	21.52	5.105
25	1	25	19.37	4.594
25	3	25	20.83	4.939

In view of this we have adhered rigidly to the experimental conditions stipulated by Powell & Whittaker and have determined the ratio of bromine to methylfurfuraldehyde over a wide range of concentrations of methylfurfuraldehyde.

Table VIII

Wt. of methylfur- furaldehyde g.	N/10 bromate absorbed ml.	Atoms bromine per mol. methylfur- furaldehyde
0.0557	22.53	4.45
0.0446	18.00	4.44
0.0334	13.7	4.50
0.0223	9.2	4.52
0.0167	6.9	4.53
0.0111	4.5	4.50
0.0056	2.2	4.30
0.0022	0.7	3.50
0.0011	0.3	2.50
0.0006	0.1	0.92

It is clear that this ratio is by no means constant as was formerly assumed, but a direct linear relationship exists between the methylfurfuraldehyde and the bromine absorbed, and this has been utilized for calculation of subsequent determinations with this method by means of a graph.

Method of Kullgren & Tyden. Kullgren & Tyden [1929] found that the first stage of the reaction between bromate and furfuraldehyde consisted of an oxidation of furfuraldehyde with accompanying formation of 2 mol. of hydrobromic acid, and they utilized this as a basis for the determination of furfuraldehyde.

These authors made the assumption that methylfurfuraldehyde behaves similarly to furfuraldehyde in absorbing 2 atoms of bromine per mol. Actually, we have found that the bromate utilization of methylfurfuraldehyde under the conditions of their titrimetric method is dependent upon the temperature, the acidity and the concentration of the methylfurfuraldehyde.

Table IX. *Kullgren & Tyden titration*

A. *Effect of temperature*

0.0079 g. methylfurfuraldehyde used in each case

Temperature °C.	N/20 bromate used ml.	Atoms bromine per mol. methylfurfur- aldehyde
18	5.78	4.02
25	5.70	3.97
33	6.04	4.21

B. *Effect of acidity*

0.0289 g. methylfurfuraldehyde used in each case; $t = 18^\circ$

Acidity (%)

0.5	17.10	3.25
0.6	20.50	3.90
0.7	20.75	3.95
0.8	21.20	4.03
0.9	21.50	4.09

C. *Effect of concentration of methylfurfuraldehyde*

Acid concentration 0.5% in each case; $t = 18^\circ$

Wt. of methylfur-
furaldehyde (g.)

0.0296	16.73	3.11
0.0236	16.47	3.84
0.0197	13.96	3.90
0.0158	11.36	3.96
0.0079	5.78	4.02
0.0039	2.93	4.13
0.0020	1.58	4.35
0.0008	0.61	4.19
0.0004	0.38	5.23
0.0002	0.21	5.78

The results in Table IX C obtained at constant temperature and acidity were adopted as standard, future results by the method being obtained by reference to a graph. We have departed from Kullgren & Tyden's technique for furfuraldehyde in one respect only: the acidity is stabilized at exactly 0.5% instead of approximately between 0.5 and 1.0%.

Comparison of foregoing methods. Having examined the relationship of the various reagents to known quantities of methylfurfuraldehyde, it next becomes of importance to study such relationships when the methylfurfuraldehyde is distilled according to the usual procedure. The modification of the salt method,

previously described, was adopted and to ensure fair standards of comparison approximately 0.05 g. methylfurfuraldehyde was used in each case, except where titrimetric methods were used, and here, in order to obviate experimental difficulties, it was necessary to use somewhat smaller amounts. Table X gives the mean of four closely agreeing determinations for each of six methods employed.

Table X

Method of determination	Wt. taken g.	Wt. in distillate g.	Apparent recovery %
Barbituric acid	0.0533	0.0347	65.1
Phloroglucinol	0.0533	0.0370	69.4
Thiobarbituric acid	0.0427	0.0314	73.4
2:4-Dinitrophenylhydrazine	0.0574	0.0436	75.9
Powell & Whittaker	0.0403	0.0312	77.4
Kullgren & Tyden	0.0318	0.0252	79.3

Since all the methods of determination have been standardized against known weights of methylfurfuraldehyde, and the known destructive effect of distillation is approximately constant a reasonably close concordance in apparent recovery might be anticipated. This is clearly not the case and hence other factors must be inducing disturbing effects. The differences become more clearly marked if rhamnose is used as the source of methylfurfuraldehyde by distillation, as shown in Table XI.

Table XI

Method of determination	Wt. of rhamnose g.	Methylfurfuraldehyde		
		Theory g.	Found g.	Recovery %
Barbituric acid	0.1002	0.0606	0.0330	54.5
Thiobarbituric acid	0.1011	0.0611	0.0365	59.7
Phloroglucinol	0.1002	0.0606	0.0388	64.0
Kullgren & Tyden	0.1001	0.0605	0.0425	70.3
Powell & Whittaker	0.0998	0.0603	0.0440	73.0
2:4-Dinitrophenylhydrazine	0.0993	0.0600	0.0450	75.0

Results by the various methods fall into two well-defined groups—the last three methods giving definitely higher results than the first three. It would seem probable, then, that some other substance, susceptible of determination by the last three methods, is produced when rhamnose and, to a less extent, when methylfurfuraldehyde is distilled.

C. Formation of acetone on distillation of rhamnose and methylfurfuraldehyde

From the foregoing it will be seen that we must look for some substance in addition to methylfurfuraldehyde which is capable of reacting under the conditions of the Powell & Whittaker, and the Kullgren & Tyden titrations and also with 2:4-dinitrophenylhydrazine.

It may be recalled that Maquenne [1889, 1] distilled *Fucus* and also rhamnose [1889, 2] with 20% sulphuric acid and obtained an aqueous liquid which on fractional distillation was stated to yield first a little acetone, followed by water and methylfurfuraldehyde. These observations appear to have been overlooked, but it appeared possible that acetone might be one of the interfering substances we were seeking and its isolation and identification were attempted as follows.

1 g. of rhamnose was distilled in the usual manner with 13.15% hydrochloric acid until 350 ml. of distillate had passed over, and the methylfurfuraldehyde was precipitated by addition of 1.5 g. thiobarbituric acid dissolved in 150 ml.

13.15% hydrochloric acid. After standing for 24 hr., the precipitate was removed by filtration through an alundum crucible and the filtrate neutralized with lead carbonate, the latter also precipitating the excess thiobarbituric acid as the insoluble lead salt. After filtration, the solution was distilled twice, the final distillate being obtained perfectly clear and colourless.

Upon addition of a solution of 2:4-dinitrophenylhydrazine in hydrochloric acid to the distillate, the latter immediately became turbid, and after standing for 24 hr., a small quantity of an orange-coloured precipitate settled out. Insufficient was obtained to permit recrystallization and determination of the melting-point.

A control experiment omitting the rhamnose but otherwise carried out exactly as above showed that the presence of some substance in the rhamnose distillate is essential to the formation of the precipitate with 2:4-dinitrophenylhydrazine. The nature of the experiment shows that the substance is not precipitable by thiobarbituric acid.

A further quantity of rhamnose was distilled and submitted to the procedure outlined, various tests being applied to the final distillate. A positive reaction was obtained in each of the following tests for acetone: (a) iodoform test [Lieben, 1870]; (b) nitroprusside test [Legal, 1888]; (c) *o*-nitrobenzaldehyde test [Penzoldt, 1885]; (d) salicylaldehyde test [Frommer, 1918].

There is therefore little doubt that acetone is produced in the acid distillation of rhamnose, although at this stage it was not possible to say whether it arose direct from rhamnose or through partial decomposition of methylfurfuraldehyde. To investigate this point, 1 g. of synthetic methylfurfuraldehyde was distilled as usual, the distillate being treated as above for rhamnose. The filtrate gave a faint turbidity with 2:4-dinitrophenylhydrazine but gave negative results in the tests for acetone enumerated above, except in the case of Frommer's reagent, where a feeble positive response was obtained.

Thus acetone probably arises as a decomposition product of methylfurfuraldehyde, but the quantity produced is extremely small compared with that formed by distillation of rhamnose.

Further experiments have demonstrated that acetone in 13.15% hydrochloric acid reacts with bromine and also yields a condensation product with 2:4-dinitrophenylhydrazine. No precipitate is furnished with barbituric acid, thiobarbituric acid or phloroglucinol.

These experiments would appear to indicate acetone as the principal, if not the sole, cause of the high apparent recoveries recorded in Tables X and XI by methods involving bromine absorption and precipitation with 2:4-dinitrophenylhydrazine. The presence of acetone, however, hardly accounts for the smaller divergencies observed in the case of the first three precipitants. The solubilities of the condensation products with phloroglucinol and barbituric acid appear to be affected to different degrees by the presence of acetone, whereas that of the thiobarbituric acid product appears to be quite unaffected.

SUMMARY

1. The distillation method of Ellett & Tollens for rhamnose determination is found to be unsatisfactory, as also the steam-distillation procedure of Pervier & Gortner.

2. Satisfactory results are obtained by a slight modification of Kullgren & Tyden's method, employing excess of salt to stabilize the acid concentration.

3. Methods for determination of the distilled methylfurfuraldehyde are reviewed; amongst precipitants, phloroglucinol and thiobarbituric acid appear to be satisfactory.

4. Titrimetric methods are only satisfactory if the results are calculated from a predetermined graph, since the relation between bromine and methylfurfuraldehyde varies with the experimental conditions.

5. Titrimetric methods and precipitation by 2:4-dinitrophenylhydrazine give appreciably higher results than precipitation by phloroglucinol and barbituric and thiobarbituric acids. This is due to the presence of acetone which is derived from rhamnose, and to a less extent from methylfurfuraldehyde, in the distillation process.

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