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

III. DETERMINATION OF METHYLPENTOSE: FACTORS INFLUENCING THE DECOMPOSITION OF METHYL- FURFURALDEHYDE DURING DISTILLATION

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(Received 31 May 1937)

INVESTIGATIONS on the carbohydrates of the cell wall, and particularly of the chemically allied group of mucilages, have pointed very clearly to the necessity of more accurate methods for the determination of methylpentoses. Thus whilst the investigations on the determination of furfuraldehyde which were described in Parts I and II of this series [Norris & Resch, 1935; Angell *et al.* 1936] were proceeding, parallel studies on methylfurfuraldehyde were instituted.

The methods for determining methylpentoses so far suggested are based on the principle that these sugars on distillation with dilute hydrochloric acid yield methylfurfuraldehyde which may be determined by precipitation or titrimetric methods. The method as originally devised by Ellett & Tollens [1905], and later extensively used by many investigators for determining methylpentoses in the presence of pentoses, appears to be subject to considerable inaccuracy for reasons to be described in a later communication, and hence it becomes desirable to seek improved methods for determining these substances where they occur together in plant materials.

Before new methods can be developed it is essential to make a propaedeutic study of the elementary principles concerned in the analytical procedure contemplated. For this purpose investigations were undertaken to ascertain the various factors responsible for the anomalous results obtained in the determination of methylfurfuraldehyde when distilled as such and also when it arises from the decomposition of methylpentoses.

EXPERIMENTAL

Preparation of methylfurfuraldehyde

For the present investigation a large supply of methylfurfuraldehyde was necessary, and this was prepared in the laboratory.

Since Stenhouse [1850] first discovered "metafural", there have been several references in the literature to the preparation of methylfurfuraldehyde from various plant materials, and more recently synthetic methods have appeared. After preliminary trials, one of the latter methods was chosen by us as a convenient means of preparing methylfurfuraldehyde. ω -Chloromethylfurfuraldehyde was prepared from sucrose, following the directions of Fischer & Neyman [1914], and subsequently reduced with stannous chloride according to

the procedure of Fenton & Gostling [1901]. Only small quantities of the aldehyde were prepared at a time and these were used for experimental purposes as soon after their preparation as was convenient.

When required for use, the sample was distilled in a semimicro-distillation apparatus, only that portion of the distillate boiling between 185–187° (corr.) being collected. The distillate was then redistilled *in vacuo*, the liquid passing over directly into a tared weighing bottle until the quantity required, as indicated by graduations on the bottle, was obtained. After reweighing, the methylfurfuraldehyde was poured into 13.15% hydrochloric acid, the bottle and the stopper thoroughly washed out with the acid, and the solution made up to volume.

The distillation procedure employed will be described completely in a subsequent communication. It will suffice here to remark that it was essentially the same as that used by Kullgren & Tyden [1929], with the exception that 22 g. salt were used instead of 20 g. as recommended by them. The distillation apparatus used in this work, unless stated to the contrary, was that which has been adopted as standard in these laboratories and described in previous communications [Angell *et al.* 1936].

In the majority of cases thiobarbituric acid was used for precipitating the methylfurfuraldehyde, chiefly because it yields precipitates of known and constant composition, which are quite insoluble, and hence methylfurfuraldehyde can conveniently be calculated by multiplying by a simple factor. However, greater accuracy can be obtained if the results are calculated by means of an equation based on experimental findings, and hence for the work discussed in this paper methylfurfuraldehyde has been calculated in this way. Full details of the method employed will be described later.

Factors influencing the decomposition of methylfurfuraldehyde

According to the conventional equation representing the formation of methylfurfuraldehyde from rhamnose, the latter should yield 60.43% of its weight of methylfurfuraldehyde.

In practice, however, the yields are always very much smaller than this, the actual quantity produced depending primarily on the mode of distillation employed and secondarily to a less extent on the amount of rhamnose used. Quotations cited in the literature would indicate that many workers in the past have assumed erroneously that not only methylpentoses, but pentoses also, always yield the same percentage weight of (methyl)furfuraldehyde under the same conditions of distillation and determination. Actually the percentage yields obtained increase with the weight of product used.

In order to study the behaviour of methylfurfuraldehyde-hydrochloric acid solutions upon distillation, freshly prepared solutions in 13.15% HCl of methylfurfuraldehyde in different concentrations were distilled according to our standard procedure for methylpentoses in the presence of 22 g. salt, and the methylfurfuraldehyde (Mef.) in the distillate was precipitated by means of thiobarbituric acid.

The results obtained (Table I) show that by this treatment the decomposition during distillation is of the order of 26–28%, the mean error of experiment being $\pm 0.8\%$.

It is evident that this decomposition is variable with the conditions of experiment and it is obviously of the first importance that the latter should be standardized for quantitative purposes. The low yield obtained is due chiefly

Table I

Wt. of Mef. g.	Wt. of ppt. g.	Mef. calc. from ppt. g.	Recovery %	Average
0-0511	0-0784	0-0369	72.2	72.6
	0-0790	0-0372	72.8	
	0-0780	0-0367	71.8	
	0-0799	0-0376	73.6	
0-1022	0-1560	0-0727	71.4	72.5
	0-1547	0-0721	70.6	
	0-1620	0-0754	73.9	
	0-1630	0-0759	74.2	
0-1487	0-2264	0-1050	70.9	71.9
	0-2263	0-1050	70.9	
	0-2354	0-1092	73.4	
	0-2312	0-1073	72.9	
0-1983	0-3068	0-1421	71.7	73.3
	0-3168	0-1467	74.0	
	0-3144	0-1456	73.4	
	0-3169	0-1468	74.0	
			Average	72.6

to two causes: (1) oxidation of methylfurfuraldehyde; (2) action of the acid, depending on its concentration.

Effect of oxidation. It has frequently been observed that methylfurfuraldehyde-hydrochloric acid solutions became discolored on standing, with deposition of brown sediments which appear to be of a humin-like nature. In order to determine whether the production of these substances involved a lowering in methylfurfuraldehyde yield, solutions of the latter were prepared and allowed to remain in bottles in the dark for a period of 2 months. At the end of this time the contents were shaken, aliquots withdrawn and methylfurfuraldehyde determined as previously, with the results shown in Table II.

Table II

Wt. of Mef. g.	Wt. of ppt. g.	Mef. calc. from ppt. g.	Recovery %	Average
0-0527	0-0537	0-0255	48.4	49.8
	0-0555	0-0264	50.1	
	0-0562	0-0267	50.7	
	0-0553	0-0263	49.9	
0-1054	0-1114	0-0521	49.4	50.0
	0-1105	0-0517	49.1	
	0-1142	0-0534	50.7	
	0-1145	0-0535	50.8	
0-2108	0-2163	0-1004	47.6	47.6
	0-2144	0-0995	47.2	
	0-2171	0-1008	47.8	
	0-2175	0-1010	47.9	
			Average	49.1

Thus the total loss of methylfurfuraldehyde arising from the period of standing and the distillation amounts to approximately 50%; loss due to the latter cause amounts to about 27% (Table I) and hence about 32% of the methylfurfuraldehyde is lost during the period of standing. That this change involves oxidation to some extent was shown by further experiments in which the distillations were carried out in an atmosphere of nitrogen. The apparatus

was necessarily modified for this type of experiment to allow of the passage of nitrogen through the boiling solutions, and the adaptor at the receiver reached below the level of the distillate in order to prevent undue loss of methylfurfuraldehyde. The use of salt in the distillation mixture had also to be abandoned in this series owing to its tendency to choke the gas delivery tube. Typical results are shown in Table III.

Table III

Wt. of Mef. g.	Wt. of ppt. g.	Mef. calc. from ppt. g.	Recovery %	Average
0.0527	0.0937	0.0440	83.5	84.3
	0.0941	0.0441	83.7	
	0.0968	0.0454	86.3	
	0.0940	0.0441	83.7	

It will be observed that the distillation of methylfurfuraldehyde in an atmosphere of nitrogen reduces the amount of decomposition to about 16%, the mean error of experiment being $\pm 1.4\%$. This would seem to imply that when methylfurfuraldehyde is distilled as is customary in air, the decomposition is due in part to oxidation, most of which must occur in the distillation flask. In this respect it is significant that the solutions which had been distilled in nitrogen were only slightly discoloured and deposited no humic substances.

The improvement in yield produced by the use of nitrogen was also illustrated in the case of rhamnose, Table IV showing comparative yields with and without the passage of nitrogen and also in the presence or absence of salt.

Table IV

Wt. of rhamnose g.	Wt. of ppt. g.	Methylfurfuraldehyde		Recovery %	Average
		Theory g.	Calc. from ppt. g.		
A. Distilled in air with salt					
0.1003	0.0784	0.0606	0.0369	61.2	61.2
	0.0783		0.0369	61.2	
B. Distilled in air without salt					
0.1003	0.0582	0.0606	0.0276	45.6	46.3
	0.0600		0.0284	46.9	
C. Distilled in nitrogen without salt					
0.1003	0.0682	0.0606	0.0322	53.2	54.3
	0.0709		0.0335	55.4	

In case B there has been no attempt to control possible oxidation or the effect of rising concentration of acid, and this shows the lowest yield. In case C oxidation has been minimized and the yield is higher; whereas in case A the highest yield is obtained although there only acid concentration and not oxidation has been controlled. Thus oxidation is clearly not the more important of the two factors under discussion, and the preponderant effect is exerted by the action of the acid during distillation.

Effect of acid concentration. In order to demonstrate beyond doubt the destructive effect of increasing concentrations of hydrochloric acid on methylfurfuraldehyde a series of experiments was conducted as follows.

30 ml. portions of a solution of 0.7778 g. methylfurfuraldehyde in 250 ml. 24% hydrochloric acid were suitably diluted to give solutions each containing 0.0927 g. of the aldehyde in acid of increasing concentrations.

All solutions were distilled in an atmosphere of nitrogen, to lessen decomposition by oxidation, at the rate of 25 ml. every 10 min. for 40 min., all the methylfurfuraldehyde having then collected in the distillate. It was not possible, of course, to maintain the acid at the initial concentration since this was liable to undergo change until the concentration of the constant boiling mixture was reached. All distillates were precipitated with solutions of thiobarbituric acid dissolved in hydrochloric acid to the appropriate concentration (Table V).

Table V

0.0927 g. of methylfurfuraldehyde used in each case

Acid conc. %	Wt. of ppt. g.	Mef. calc. from ppt. g.	Recovery %
8	0.1813	0.0843	90.9
12	0.1656	0.0771	83.2
16	0.1300	0.0607	65.5
20	0.0927	0.0435	46.9
24	0.0591	0.0280	30.2

Experiments on exactly similar lines were conducted in the case of rhamnose, the results being shown in Table VI and discussed at a later stage.

Table VI

0.1992 g. rhamnose used in each case, equivalent to 0.1203 g. methylfurfuraldehyde (conventional)

Acid conc. %	Wt. of ppt. g.	Mef. calc. from ppt. g.	Recovery %
2	0.1675	0.0779	64.7
6	0.1589	0.0740	61.5
8	0.1516	0.0706	58.7
12	0.1377	0.0642	53.3
16	0.1229	0.0574	47.7
20	0.1021	0.0478	39.7
24	0.0835	0.0393	32.7

The necessity for stabilizing the concentration of acid at as low a concentration as possible is thus effectively demonstrated. That the addition of salt to the acid on the lines suggested by Kullgren & Tyden [1929] has a salutary effect was shown by determination of the distillation factors in experiments conducted without and with the addition of salt.

The distillation factor is the ratio between the weight of methylfurfuraldehyde in the vapour and that in the solution at any given time during the distillation, and may be expressed as:

$$k = \frac{V}{v} \times \log_n \frac{a}{a-x},$$

where V = mean volume of "distilland" in flask,

v = volume of distillate withdrawn at any particular stage in the distillation,

a = total weight of methylfurfuraldehyde remaining to be distilled,

$a-x$ = wt. of methylfurfuraldehyde in v ml. of distillate.

In the case of furfuraldehyde, Kullgren & Tyden found the value of k to be approximately constant.

In the present instance the methylfurfuraldehyde in small aliquots of the distillate was determined by titrimetric means, with the results shown in Table VII.

Table VII

I. *Ellett & Tollens distillation*

A. Total original methylfurfuraldehyde, 0.0285 g.

Distillate sample <i>v</i> ml.	Weight of methylfurfuraldehyde			Vol. of distilland <i>V</i> ml.	$k = \frac{V}{v} \times 2.303 \log_{10} \frac{a}{a-x}$
	<i>x</i> g. in <i>v</i> ml.	<i>a</i> g.	<i>a</i> - <i>x</i> g.		
5	0.0158	0.0285	0.0127	97.5	15.8
5	0.0075	0.0127	0.0052	92.5	16.5
10	0.0011	0.0052	0.0041	85.0	2.0
10	0.0030	0.0041	0.0011	87.5	11.5
10	0.0009	0.0011	0.0002	90.0	15.3
10	0.0002	0.0002	0	80.0	—

B. Total original methylfurfuraldehyde, 0.0300 g.

5	0.0170	0.0300	0.0130	97.5	16.3
5	0.0072	0.0130	0.0058	92.5	15.3
10	0.0021	0.0058	0.0037	85.0	3.8
10	0.0027	0.0037	0.0010	87.5	11.4
10	0.0008	0.0010	0.0002	90.0	14.5
10	0.0002	0.0002	0	80.0	—

II. *Modified Kullgren & Tyden distillation*

A. Total original methylfurfuraldehyde, 0.0491 g.

5	0.0333	0.0491	0.0158	97.5	22.1
5	0.0088	0.0158	0.0070	92.5	15.1
10	0.0055	0.0070	0.0015	85.0	13.1
10	0.0010	0.0015	0.0005	87.5	9.6
10	0.0005	0.0005	0	90.0	—

B. Total original methylfurfuraldehyde, 0.0468 g.

5	0.0323	0.0468	0.0145	97.5	22.8
5	0.0082	0.0145	0.0063	92.5	15.4
10	0.0048	0.0063	0.0015	85.0	12.2
10	0.0010	0.0015	0.0005	87.5	9.6
10	0.0005	0.0005	0	90.0	—

It will be observed that in each case the time taken for the methylfurfuraldehyde to distil was extremely short, all having passed over in the first 50 ml. of distillate.

When no salt is present during the distillation, the distillation factor is not constant, and its minimum value is so low as to suggest that considerable decomposition is being effected at the stage when the acid concentration has begun to rise.

On the other hand, when the acid concentration is maintained at 13.15% by the addition of salt the methylfurfuraldehyde distils over much faster and the value of the distillation factor is considerably higher, indicating that less decomposition is occurring, but in this case also it does not remain constant, for it shows a steady decrease in value until all the methylfurfuraldehyde has passed over. However, its minimum value is never so low as to suggest that considerable decomposition is taking place at any one period. The gradual change in the distillation factor may be attributed to the different periods for which the methylfurfuraldehyde in each distillation fraction is exposed to the destructive action of the hot acid.

Thus, where compromise is in any circumstance unavoidable, the use of salt to stabilize the concentration of acid at 13.15% would appear to offer a satisfactory solution.

DISCUSSION

Kröber & Rimbach [1902] found that the yields of furfuraldehydephloroglucide from arabinose and xylose reached a maximum when the sugars were distilled with acid of 16% concentration. With more concentrated acid, the yields decreased, owing to greater destruction of the furfuraldehyde; with acids of lower concentration, the yields were also lower, since the acid was unable to break down the sugars completely. Yet with rhamnose, on the other hand, we have found that the maximum yield of methylfurfuraldehyde was obtained with acid of 2% concentration. Incidentally, the yield with 2% acid was actually higher than that obtained from the corresponding quantity of sugar when the acid concentration was maintained throughout at 13.15%. This must signify that, as compared with the pentoses, rhamnose is very easily attacked by hydrochloric acid and the greater part of the methylfurfuraldehyde is formed during the initial stages of the reaction. That rhamnose, under the usual conditions of distillation, yields less than the theoretical amount of methylfurfuraldehyde is due very largely to decomposition of the latter for the various reasons outlined. But even after correcting for the amount of methylfurfuraldehyde that is destroyed during distillation, there still appears to be a loss due to some other cause, for theoretical values are never obtained.

Our values for the distillation factor show that when methylfurfuraldehyde is distilled the amount decomposed depends upon the time of exposure to the destructive action of the acid. But when a methylpentose is distilled, the concentration of the methylfurfuraldehyde produced at each corresponding stage of the distillation will depend upon its rate of formation from the methylpentose and therefore the amount decomposed will be different from that destroyed when a methylfurfuraldehyde-hydrochloric acid solution alone is distilled.

To a certain extent, experimental evidence is available to support this statement. By interpolation of the results given in Tables V and VI it can be calculated that on distillation with acid of 23.5% concentration the yield of methylfurfuraldehyde from rhamnose will be the same as that recovered by distillation of methylfurfuraldehyde alone, provided that the quantities of each product used correspond to those given in the tables. Presumably with acid of this concentration, the rate of formation of methylfurfuraldehyde from rhamnose is considerably accelerated, and the average time for the influence of the acid on the methylfurfuraldehyde will be very little different from that when methylfurfuraldehyde alone is distilled under the same conditions. It would appear, therefore, that in this case rhamnose could yield its theoretical amount of methylfurfuraldehyde.

Under normal conditions of distillation, however, such is not the case, the relatively slow transformation into methylfurfuraldehyde being accompanied by simultaneous degradation of the rhamnose itself into humic substances, and the conventional reaction

probably takes place via a series of intermediate products as suggested by Votocek & Rac [1929] and Hurd & Isenhour [1932], all of which at the same time may be capable of giving rise to other products besides methylfurfuraldehyde, depending upon their rate of formation and subsequent time of exposure

to the destructive action of the acid before being ultimately transformed into methylfurfuraldehyde.

This varying degree of stability towards hydrochloric acid must account, in no small measure, for the lower yield of methylfurfuraldehyde obtained by Mayer & Tollens [1907] from fucose and by Votocek & Rac [1929] from 2-keto-rhamnose, in comparison with that obtainable from rhamnose.

The production of furfuraldehyde from pentoses has been considered to depend in part on the stereochemical configuration of the molecule [Bott & Hirst, 1932; Hurd & Isenhour, 1932]. In the present instance we were able to substantiate this view by plotting distillation curves for xylose, rhamnose and arabinose. Such curves indicate the rate of production of (methyl)furfuraldehyde from the sugar and it was found that this followed the order given.

SUMMARY

1. When methylfurfuraldehyde is distilled in hydrochloric acid solution by the usual Tollens procedure, about 27 % of the aldehyde is destroyed.
2. The loss is due in part to oxidation, since an improved yield is obtained if distillation is carried out in a nitrogen atmosphere.
3. The preponderant cause of loss is the action of the acid which becomes more marked as its concentration increases.
4. A study of distillation factors shows that it is more satisfactory to distil in the presence of salt which stabilizes the acid concentration.
5. The yield of methylfurfuraldehyde from methylpentoses is not only influenced by acid concentration and oxidation but also varies with the stereochemical configuration of the sugar. Rhamnose appears to decompose more readily than the pentoses.

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