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

V. DETERMINATION OF METHYLPENTOSE SINGLY AND IN SIMPLE MIXTURES

BY CHARLES ROY MARSHALL
AND FREDERICK WALTER NORRIS

From the Department of Industrial Fermentation, University of Birmingham

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THE fundamentals of the distillation and precipitation procedures involved in the determination of methylpentoses were investigated and discussed in Parts III and IV of this series [Marshall & Norris, 1937] in which it was shown that under the usual conditions of distillation a large and varying amount of methylfurfuraldehyde was destroyed, partly by oxidation but mostly by action of the acid used. The effects of distillation may be controlled within much narrower limits if the concentration of the acid is stabilized by the addition of excess of sodium chloride. Titrimetric methods of determining methylfurfuraldehyde in the distillate were found to be unsatisfactory especially if mixtures containing pentoses and hexoses in addition to methylpentoses were under examination. Of the usual precipitants employed, phloroglucinol, thiobarbituric acid and possibly barbituric acid were found to be fairly satisfactory but the discovery of acetone in distillates from rhamnose, for example, rendered undesirable the use of 2:4-dinitrophenylhydrazine and also of titrimetric methods.

In the present communication we seek to establish relationships between methylfurfuraldehyde and rhamnose and the precipitates obtained with the first three reagents above, and by comparison of standard errors to determine the most suitable precipitant to employ. The method of treatment was precisely similar to that described for pentoses in Part II of this series [Angell *et al.* 1936] and the strictures on standardization and the need of individual determination of relationships stressed in that paper remain valid in the present instance. Furthermore, since, when working with substances giving rise to methylpentoses it is necessary to employ salt with the acid in distillation, the relationships between furfuraldehyde, the pentoses and their precipitants under these conditions have been determined anew and found to differ from those obtained when acid only is used as distillation medium. Finally, attempts have been made to determine relationships for various simple pentose and methylpentose mixtures.

EXPERIMENTAL

The distillation procedure employed was based on the findings of Parts III and IV [Marshall & Norris, 1937] and the apparatus used was described in Part II [Angell *et al.* 1936]. The exact procedure may be summarized as follows.

(1939)

A weighed quantity of the sample was distilled with 100 ml. 13.15 % hydrochloric acid in the presence of 22 g. sodium chloride. Distillation was maintained at the rate of 25 ml. in 10 min., and acid was added accordingly until 350 ml. of distillate were obtained.

All samples were of the highest purity attainable and were added as aliquots of a standard solution in 13.15 % hydrochloric acid, except in the case of pectolic acid, which, being insoluble, was added in weighed portions. Where methylfurfuraldehyde was determined directly a known weight of the freshly distilled liquid was made up to 350 ml. with 13.15 % acid.

Precipitation was carried out according to the methods for each particular reagent given in Part IV.

In the following tables are shown the weights of methylfurfuraldehyde, anhydrosorhamnose, furfuraldehyde, anhydroarabinose, anhydroxylose and pectolic anhydride, as carbon dioxide (indicated by *MeF*, *R*, *F*, *A*, *X* and *C_p*, respectively) and the corresponding weights of precipitate with phloroglucinol, thiobarbituric acid and barbituric acid (*P*, *T* and *B*) from which the equations shown later were calculated. Where four results only are given, each of these represents the average of four closely agreeing determinations, sixteen experiments being performed in all.

Table I. *Methylfurfuraldehyde*

Wt. of <i>MeF</i> g.	Wt. of ppt. <i>P</i> g.	Wt. of ppt. <i>T</i> g.
0.0279	0.0430	0.0608
0.0575	0.1055	0.1213
0.1149	0.2222	0.2467
0.1671	0.3209	0.3622

Table II. *Anhydrosorhamnose*

Wt. of <i>R</i> g.	Wt. of ppt. <i>P</i> g.	Wt. of <i>R</i> g.	Wt. of ppt. <i>T</i> g.	Wt. of <i>R</i> g.	Wt. of ppt. <i>B</i> g.
0.0404	0.0246	0.0405	0.0376	0.0402	0.0150
0.0605	0.0476	0.0801	0.0784	0.0804	0.0568
0.0804	0.0669	0.0801	0.0766	0.1212	0.0908
0.0800	0.0664	0.0811	0.0777	0.1609	0.1316
0.1205	0.1061	0.1203	0.1161	—	—
0.1602	0.1398	0.1208	0.1158	—	—
—	—	0.1605	0.1577	—	—

Table III. *Furfuraldehyde*

Wt. of <i>F</i> g.	Wt. of ppt. <i>P</i> g.	Wt. of <i>F</i> g.	Wt. of ppt. <i>T</i> g.
0.0252	0.0451	0.0212	0.0488
0.0505	0.0966	0.0423	0.1008
0.1009	0.1958	0.0982	0.2373
0.1514	0.2930	0.1269	0.3181
—	—	0.1692	0.4244

Table IV. *Anhydroarabinose*

Wt. of <i>A</i> g.	Wt. of ppt. <i>P</i> g.	Wt. of <i>A</i> g.	Wt. of ppt. <i>T</i> g.	Wt. of <i>A</i> g.	Wt. of ppt. <i>T</i> g.
0.0438	0.0409	0.0441	0.0526	0.0441	0.0416
0.0876	0.0885	0.0884	0.1111	0.0884	0.0985
0.1297	0.1344	0.1324	0.1744	0.1324	0.1475
0.1785	0.1875	0.1763	0.2287	0.1763	0.1990

Table V. *Anhydroxylose*

Wt. of <i>X</i> g.	Wt. of ppt. <i>P</i> g.	Wt. of <i>X</i> g.	Wt. of ppt. <i>T</i> g.	Wt. of <i>X</i> g.	Wt. of ppt. <i>B</i> g.
0.0442	0.0507	0.0444	0.0679	0.0444	0.0543
0.0884	0.1083	0.0891	0.1399	0.0882	0.1151
0.1326	0.1657	0.1315	0.2081	0.1316	0.1727
0.1769	0.2199	0.1757	0.2763	0.1757	0.2396

Table VI. *Pectolic anhydride, as carbon dioxide*

Wt. of <i>C_p</i> g.	Wt. of ppt. <i>P</i> g.	Wt. of <i>C_p</i> g.	Wt. of ppt. <i>T</i> g.	Wt. of <i>C_p</i> g.	Wt. of ppt. <i>B</i> g.
0.0219	0.0349	0.0224	0.0465	0.0239	0.0396
0.0227	0.0363	0.0224	0.0470	0.0233	0.0378
0.0233	0.0365	0.0237	0.0510	0.0242	0.0389
0.0246	0.0387	0.0223	0.0475	0.0466	0.0845
0.0463	0.0767	0.0420	0.0904	0.0464	0.0831
0.0466	0.0771	0.0466	0.0999	0.0439	0.0792
0.0471	0.0773	0.0422	0.0922	0.0449	0.0800
0.0474	0.0773	0.0455	0.0977	0.0689	0.1286
0.0690	0.1150	0.0695	0.1515	0.0692	0.1290
0.0685	0.1130	0.0679	0.1475	0.0681	0.1266
0.0703	0.1173	0.0668	0.1466	0.0686	0.1281
0.0686	0.1134	0.0689	0.1515	—	—

The relationships derived from the above experimental results are expressed in the following equations, numbered *seriatim* after those given in Part II.

(i) *Phloroglucinol as precipitant*:

- (9) Methylfurfuraldehyde: $MeF = 0.5P + 0.0053 \pm 0.0017$.
- (10) Anhydrorhamnose: $R = 1.0433P + 0.0118 \pm 0.00032$.
- (11) Furfuraldehyde: $F = 0.5098P + 0.0016 \pm 0.00079$.
- (12) Anhydroarabinose: $A = 0.9187P + 0.00625 \pm 0.00006$.
- (13) Anhydroxylose: $X = 0.783P + 0.00397 \pm 0.00103$.
- (14) Pectolic anhydride, as carbon dioxide: $C_p = 0.5888P + 0.0015 \pm 0.00032$.

(ii) *Thiobarbituric acid as precipitant*:

- (15) Methylfurfuraldehyde: $MeF = 0.4605T + 0.00078 \pm 0.0010$.
- (16) Anhydrorhamnose: $R = 1.0074T + 0.00267 \pm 0.0011$.
- (17) Furfuraldehyde: $F = 0.3922T + 0.00277 \pm 0.00123$.
- (18) Anhydroarabinose: $A = 0.7442T + 0.00485 \pm 0.00188$.
- (19) Anhydroxylose: $X = 0.6293T + 0.0013 \pm 0.00044$.
- (20) Pectolic anhydride, as carbon dioxide: $C_p = 0.45T + 0.00117 \pm 0.00038$.

(iii) *Barbituric acid as precipitant*:

- (21) Anhydrorhamnose: $R = 1.048B + 0.0236 \pm 0.0027$.
- (22) Anhydroarabinose: $A = 0.8446B + 0.00755 \pm 0.00197$.
- (23) Anhydroxylose: $X = 0.7122B + 0.0064 \pm 0.0019$.
- (24) Pectolic anhydride, as carbon dioxide: $C_p = 0.5025B + 0.00436 \pm 0.00014$.

Relative accuracy of the three precipitants. The relative accuracy of the results summarized in the foregoing equations is proportional to the squares of the standard errors. Examination shows that the relative accuracy of barbituric acid as a precipitant is very low, except in the case of pectolic anhydride, which is quite anomalous, and in general this precipitant appears to be the least satisfactory of the three.

Comparison of the standard errors in the above series with those obtained for the series in Part II (wherein the distillation was carried out without added salt) shows that these are of the same order; but there is a balance in favour of the

salt distillation method. In general, whilst this is essential for products yielding methylfurfuraldehyde, it is desirable also for those yielding only furfuraldehyde. Moreover, the two sets of equations were submitted to the "t" test of Fisher [1932] and in every case it was found that there was a significant difference between the coefficients of the equations derived from data by the salt distillation method and those of the corresponding equations obtained where the distillation method did not involve added salt.

Mixtures containing methylpentoses. In general, the analysis of mixtures containing methylpentoses in addition to pentoses, uronic acids and/or hexoses, is more complex than that of other mixtures described in earlier parts of this series. The errors accumulate very rapidly as the number of constituents increases, and with the methods of analysis at present available it is not profitable to examine systematically all the possible mixtures.

In the meantime, the remaining notes based on laboratory experience may be of use in the case of the simpler binary mixtures.

Mixtures of methylpentose and uronic acid. This case should present little difficulty and may be treated on similar lines to those indicated in Part II for mixtures of arabinose and uronic acid, for example. Artificial mixtures distilled (with addition of salt) and precipitated with phloroglucinol (or thiobarbituric acid) would yield results whereby the general equation $MeP = aP + bC + c$ could be deduced. Since P and C are known (P = wt. of precipitate, C = wt. of carbon dioxide from uronic acid), MeP is readily calculated. The effect of hexoses on the determination could also be shown as already described.

Mixtures of methylpentose and hexose. Mixtures of this type also may be treated without great difficulty. The presence of hexose is likely to influence the final result in two ways: (a) it may increase or decrease the yield of methylfurfuraldehyde from the methylpentose; (b) it may yield appreciable amounts of ω -hydroxymethylfurfuraldehyde which is precipitated by phloroglucinol. The first effect will be fully compensated by mathematical treatment of the results obtained from the usual series of artificial mixtures. This also applies to the second effect which may, however, be rendered negligible by the use of thiobarbituric acid which does not precipitate the hydroxy-derivative.

Mixtures of methylpentose and pentose. This is the commonest type of binary mixture and the one most difficult of analysis, since there appears still to be no really satisfactory method of determination. Experiments were carried out with artificial mixtures of rhamnose and arabinose, using phloroglucinol and thiobarbituric acid as precipitants with the following results.

Table VII

Anhydro-arabinose g.	Anhydro-rhamnose g.	Phloroglucide		Thiobarbituric acid ppt.	
		Found g.	Calculated* g.	Found g.	Calculated† g.
0.0615	0.0563	0.1028	0.1028	0.1284	0.1282
0.0615	0.1126	0.1553	0.1567	0.1801	0.1852
0.0615	0.1689	0.2056	0.2107	0.2231	0.2411
0.1230	0.0563	0.1710	0.1697	0.2093	0.2108
0.1230	0.1126	0.2200	0.2237	0.2526	0.2679
0.1230	0.1689	0.2689	0.2776	0.3128	0.3238
0.1845	0.0563	0.2391	0.2366	0.2835	0.2934
0.1845	0.1126	0.2875	0.2906	0.3452	0.3505
0.1845	0.1689	0.3268	0.3445	0.3837	0.4064

* The figures in this column are the sums of the weights of phloroglucide obtained from arabinose and rhamnose according to equations (10) and (12).

† Similarly calculated from equations (16) and (18).

Comparison of the "found" and "calculated" values shows that the yield of precipitate obtained from the mixture is generally lower than the sum of the yields to be anticipated from the individual components, that the discrepancy is greater for increasing weights of precipitate, and that it is usually greater for the thiobarbituric acid precipitate than for the phloroglucide.

Apart, then, from the objections already raised to the method of Ellett & Tollens [1905], this fact indicates a further inaccuracy. For even supposing the original distillation procedure were satisfactory, and the separation of furfuraldehydphloroglucide from methylfurfuraldehydphloroglucide by alcohol extraction absolute, the fact remains that the residual phloroglucide is not a true measure, by the factors originally suggested, of the arabinose in the mixture.

From the results of Table VII equations of the usual type have been deduced:

$$(25) \ A = 0.00637 + 0.9441 P - 0.806 R \pm 0.0033.$$

$$(26) \ A = 0.00215 + 0.7641 T - 0.675 R \pm 0.0041.$$

The standard errors for the determination of the mixtures are much higher than those for single substances. In view of these errors the equations may not be used in combined form for determining A and R ; moreover, they both express the results of the same fundamental process, namely, precipitation.

DISCUSSION

At the present stage in our investigations we have endeavoured to show that by suitable stabilization and control of the distillation procedure, it is possible to determine methylpentoses, of which rhamnose has been utilized as the prototype, with greater accuracy than hitherto. Where the methylpentose is one member of a binary mixture it is still possible to determine this component provided that the other component is susceptible of independent determination, as in the case of a uronic acid. We are unable, at present, to suggest an absolute method for determining pentose and methylpentose in admixture, although investigations on this point are still proceeding. An approximate assay may be made as follows: Parallel distillations (salt-hydrochloric acid) are made, the distillates being precipitated with phloroglucinol and with thiobarbituric acid. The phloroglucide is extracted with alcohol and the residual phloroglucide calculated approximately from the equations (e.g. for arabinose, equation (12)). Equation (18) is then used to calculate the weight of thiobarbituric acid precipitate corresponding to the arabinose. The difference between this and the total thiobarbituric acid precipitate gives that due to rhamnose (e.g.) which may then be calculated by means of equation (16). The cross calculation to thiobarbituric acid precipitate is made since the method could then be applied when hexose is also present. Admittedly the results are still only approximate and subject to the strictures previously mentioned, yet they are still likely to be more accurate than those by the earlier methods. For, by standardizing and stabilizing the distillation conditions, the extent of decomposition of the methylfurfuraldehyde is strictly controlled and the conditions approach very closely those under which the original determinations for standardization were made.

It is opportune at this point to discuss briefly one or two points which have arisen since the present series was commenced. It may be emphasized that our experiments have dealt solely with single substances and simple mixtures of pentose, methylpentoses and uronic acids as they most frequently occur in cell-wall products. We believe that the accurate proximate analysis of natural products such as straw, wood etc., is quite impossible with existing methods of analysis and that the customary statement that a given substance yields a

certain percentage of furfuraldehyde is virtually meaningless. It is not yet possible to state accurately the proportions of furfuraldehyde derived from pentose and uronic acid respectively from such complex substances, owing to the many factors exerting disturbing effects on the results. In dealing with simple mixtures, phloroglucinol still appears to be the most satisfactory precipitant, whilst thiobarbituric acid is useful in some cases. Nevertheless, it is not surprising that Campbell & Smith [1937] find difficulties in the use of the latter in the analysis of woods, since the distillate from woods must contain a most complex mixture of substances, any or all of which may have a disturbing effect on the results obtained and may also be responsible in part for the physical difficulties they encountered.

The above workers appear to be under the impression that we have adversely criticized the work of Kröber [1901] although this was not our intention, since Kröber's work is to be regarded as classic. Our criticisms were levelled rather at the modern practice of the method in that two essential features of the original method appear to have been overlooked: (a) Kröber's tables were constructed for single substances in pure condition; they have since been applied without correction to the most diverse and complex mixtures; (b) his apparatus and method were standardized but have since been modified by workers who nevertheless still employ his original tables. Campbell & Smith themselves use an apparatus which is not identical with that of Schorger [1926] and this again differs from that of Kröber. Nevertheless it is readily admitted that errors introduced in this manner are of no great significance as compared with those necessarily involved in the analysis of woods (in contrast to that of single substances or simple binary mixtures).

Iddles & French [1936] have studied the determination of methylfurfuraldehyde and conclude that phloroglucinol, thiobarbituric acid and 2:4-dinitrophenylhydrazine are equally satisfactory as precipitants. In Part IV of this series we found that the latter was unsuitable, and it may be pointed out that the results of Iddles & French were based on direct precipitation of pure methylfurfuraldehyde and not on that of the distillates arising in the usual method of determination.

SUMMARY

1. Using the improved distillation methods advocated in earlier papers of the series, the relationships between furfuraldehyde, arabinose, xylose, pectolic anhydride, methylfurfuraldehyde and rhamnose, and the precipitates formed by the distillates with phloroglucinol, thiobarbituric acid and barbituric acid are determined.

2. Phloroglucinol is found to be the best precipitant; thiobarbituric acid is also suitable in some cases, while barbituric acid is the least satisfactory.

3. Suggestions are made with reference to the analysis of simple mixtures of methylpentoses with pentoses and/or uronic acids. The analysis of methylpentose and pentose mixtures is not yet satisfactory.

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