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

I. THE RELATION BETWEEN URONIC ANHYDRIDE CONTENT AND FURFURALDEHYDE YIELD.

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THIS communication has been planned as the first of several dealing with problems in the analysis of the carbohydrate constituents of the cell wall of plants, with special reference to pectin and the hemicelluloses. Such substances yield on hydrolysis hexoses, pentoses and uronic acids, and the most important reaction employed in their proximate analysis involves distillation with 12 % hydrochloric acid, whereby hexose fractions yield a small quantity of hydroxymethylfurfuraldehyde, pentose and methylpentose fractions yield furfuraldehyde and methylfurfuraldehyde, and similarly the uronic acid fraction yields furfuraldehyde, but in addition undergoes decarboxylation with production of carbon dioxide. The products utilised in this publication are pure uronic acids, or uronic acid derivatives, and the analytical complications due to the presence of hexoses and methylpentoses do not arise.

In order, then, to determine the amount of the separate constituents present in any particular cell wall substance, it is necessary to determine, on distillation with 12 % hydrochloric acid:

- (i) The total carbon dioxide produced.
- (ii) The total furfuraldehyde produced.
- (iii) The total methylfurfuraldehyde produced (if methylpentoses are present).

In order to calculate the final analysis certain data must be accurately known:

- (i) The normal percentage yield of carbon dioxide from the uronic acid.
- (ii) The normal percentage yield of furfuraldehyde from the uronic acid.
- (iii) The normal percentage yield of furfuraldehyde from the pentose.
- (iv) The normal percentage yield of methylfurfuraldehyde from the methylpentose.
- (v) The effect of each or all of the above-mentioned constituents, and of hexoses, if present, on the normal percentage yields enumerated.

The relation between uronic acid content and carbon dioxide yield would appear to be the only factor which is known with absolute accuracy. Fortunately the yield of carbon dioxide appears to be theoretical, although it may be mentioned that in dealing with pure uronic acids the evaluation by carbon dioxide determination is usually very slightly lower than that obtained by direct titration. However, the method is not an empirical one and the difficulties have been entirely experimental. It is now safe to say that the carbon dioxide produced on distillation with 12 % hydrochloric acid can be accurately estimated and the uronic acid content calculated directly.

The literature dealing with the production of furfuraldehyde from pentoses and pentosans is extremely voluminous and it is unnecessary to discuss the history of the adaptation of the method to quantitative estimation. It is sufficient to say that the use of phloroglucinol as a precipitant for the distilled furfuraldehyde is still probably the most satisfactory way of estimating the latter and that the publications of Tollens and his co-workers, particularly Kröber [1901], comprise the most complete accounts of the method. In the present communication the methods laid down by these workers have been followed with very little modification, and Kröber's tables have been used for purposes of calculation.

The primary object in publishing this communication is to draw the attention of chemists working in this field to a fatal misconception of the yield of furfuraldehyde from uronic acids which has arisen in recent years. This appears to be due to the acceptance of statements made by Nanji, Paton and Ling [1925], who themselves appear to have misread earlier publications by Lefevre and Tollens [1907]. Thus, the former state that the yield of furfuraldehyde from uronic acid is 31 % of the theoretical and that galacturonic anhydride units yield 16.66 % of their weight of furfuraldehyde. It is wellnigh impossible to reconcile these statements with a correct interpretation of the results of Lefevre and Tollens, who employed glycuronic lactone and derivatives of glycuronic acid, such as euxanthic acid, as reference substances and clearly demonstrated that the weight of furfuraldehyde phloroglucide produced corresponded to one-third of the weight of glycuronic anhydride present. A recalculation of their results, involving merely the substitution of the weight of furfuraldehyde for that of the precipitate by means of Kröber's tables, showed that as an average of eleven determinations on four different products, the furfuraldehyde yield was 18.73 % of the weight of glycuronic anhydride, or that glycuronic anhydride was given by furfuraldehyde $\times 5.34$. The data of Lefevre and Tollens therefore showed that the yield of furfuraldehyde from glycuronic anhydride was 34.34 % of theory.

Incidental references to the subject by Ehrlich and Schubert [1929] in which experimental results of the furfuraldehyde yield from galacturonic acid are given, coupled with our own present findings, fully confirm that the original factors of Lefevre and Tollens are more correct than those of Nanji *et al.* [1925].

EXPERIMENTAL.

Determination of uronic acids by decarboxylation.

The most satisfactory method for this determination is that employed by Dickson *et al.* [1930]. The decomposition of the material is effected by means of 12 % hydrochloric acid contained in a flask fitted with a reflux condenser, to which is attached a trap and a small Büchner flask carrying a Truog tower. The reaction flask is also connected on the other side to a train of wash-bottles, towers and U-tubes containing 50 % caustic potash solution, soda-lime, calcium chloride *etc.*, in order that the stream of air which is drawn through the entire apparatus may be free from carbon dioxide. On heating the reaction mixture to 140–145°, the evolved carbon dioxide is drawn through a known volume of standard barium hydroxide contained in the Truog tower. When decomposition is complete, the carbon dioxide produced is estimated by back-titration with standard acid, using phenolphthalein as indicator.

Certain precautions have been found necessary if satisfactory yields of carbon dioxide are to be obtained. Prior to the initiation of the decarboxylation it is essential to ensure that all carbon dioxide has been swept out of the apparatus by means of the current of carbon dioxide-free air. Carbon dioxide is frequently adsorbed by the calcium chloride used to scrub the air before it passes into the reaction flask. With regard to the Truog tower, a definite technique must be

adopted in adding the standard barium hydroxide, and we have found it desirable to fit a 30 ml. cylindrical dropping funnel to the top of the tower. This in turn is fitted with a soda-lime tube and a second glass tube bearing a small piece of pressure-tubing and a screw clip. The funnel is previously filled with carbon dioxide-free air, and standard barium hydroxide is added from an automatic pipette whereby contamination with carbon dioxide is entirely obviated. At the commencement of the actual decarboxylation, the baryta is allowed to run slowly down the tower, and it is advisable always to ensure that some of the baryta falls into the flask which carries the tower. Whilst the baryta is slowly flowing into the tower, the temperature of the glycerol-bath which carries the reaction flask is slowly raised, the current of air being regulated as necessary. The temperature of the glycerol-bath must be maintained at 140–145°. We have found that the minimum time required for completion of the reaction is 5 hours, but a longer period is frequently necessary.

From time to time blank determinations have been carried out in order to keep a satisfactory check on the apparatus, but always the correction has proved negligible. On actual trial with pure uronic acids the apparatus has given results which differ from the theoretical only by the same negligible margin.

The following reference substances were used for the purpose of the present paper:

(1) *Galacturonic acid monohydrate*, $C_6H_{10}O_7 \cdot H_2O$. This product was prepared by synthesis from galactose and the only impurity present in the three samples A, B and C was water. Further drying was not attempted owing to possible decomposition of the samples and the actual content of galacturonic acid was determined by estimation of carbon dioxide on decomposition with 12 % hydrochloric acid or by direct titration using phenolphthalein. The theoretical yield of carbon dioxide from galacturonic acid monohydrate is 20.76 %.

(2) *Pectolic acid*. This product was employed since it is an example of a polygalacturonic acid. It was prepared by Ehrlich and Schubert [1929] and Ehrlich and Guttmann [1933] by partial hydrolysis of pectin with 5 % hydrochloric acid, and their methods were followed closely by us. Ehrlich called the product "Pektolsäure", which we have translated as pectolic acid, and according to him its constitution may be represented by a four-membered ring consisting solely of galacturonic anhydride units, together with one molecule of water. Its empirical formula is thus $C_{24}H_{32}O_{24} \cdot H_2O$. The theoretical yield of carbon dioxide from a substance of this formula is 24.39 %, equivalent to 107.54 % galacturonic acid.

(3) *Euxanthic acid*. The theoretical yield of carbon dioxide from euxanthic acid is 10.43 %, equivalent to 45.7 % glycuronic acid.

The analysis of these reference substances is shown in Table I.

Table I.

Sample	Carbon dioxide % found	Anhydride $C_6H_8O_6$ % calc.	Acid, $C_6H_{10}O_7$ %		Purity (based on CO_2 det.)
			Calc.	Found (titration)	
Galacturonic acid A	19.75	79.00	87.08	87.50	93.13
Galacturonic acid B	20.67	82.68	91.14	91.54	99.56
Galacturonic acid C	20.11	80.44	88.67	88.67	96.87
Pectolic acid	23.64	94.56	104.23	—	97.01
Euxanthic acid	10.43	41.72	45.70	—	100.00

The amount of galacturonic acid C available was small; the figures quoted for this sample are calculated from the results of direct titration only.

Determination of furfuraldehyde.

A definite weight of material and 100 ml. of 12 % hydrochloric acid are placed in a flask of 500 ml. capacity connected by a rubber bung to a Liebig's condenser which carries an adaptor for collecting the distillate in a conical flask. The apparatus is set up in duplicate and the reaction flasks are placed side by side in a bath of glycerol, each flask being immersed in the glycerol exactly to the depth of the 100 ml. acid within. The temperature of the bath is raised to 170° and this temperature is closely adhered to throughout the distillation, which proceeds at the same rate in each flask. As soon as the acid reaches boiling-point, 60 ml. of acid are added, and a similar quantity at 20-minute intervals until six additions have been made. The distillation should be complete in 2 hours and the furfuraldehyde is then precipitated as phloroglucide, by the method suggested by Browne [1912]. 11 g. of phloroglucinol are dissolved in 300 ml. of boiling 12 % hydrochloric acid and added to a further 1200 ml. of acid. The solution is allowed to stand for at least a week and is then ready for use after filtration. The phloroglucinol used should be pure and, particularly, free from diresorcinol; in this respect it was found that AnalaR phloroglucinol was preferable.

40 ml. of this filtered solution are added to the distillate, which should then occupy 400 ml., any slight deficiency being made up with 12 % acid. The mixture is allowed to stand overnight and is then filtered through a weighed Jena sintered glass crucible (grade 1 G. 4), washed with 100 ml. of distilled water, dried at 100° for 3 hours and again weighed.

Table II. *The yield of furfuraldehyde from uronic compounds.*

Wt. taken g.	Equiv. to		Wt. of phloro- glucide g.	Wt. of furfur- aldehyde g.	Yield of furfuraldehyde		
	Uronic anhydride g.	Uronic acid g.			Based on		As per- centage of theory
					Anhydride %	Acid %	
Galacturonic acid A.							
0.2017	0.1593	0.1756	0.0686	0.0383	24.04	21.81	44.07
0.2048	0.1618	0.1783	0.0657	0.0368	22.75	20.64	41.70
0.2564	0.2016	0.2233	0.0858	0.0473	23.35	21.18	42.80
0.2752	0.2174	0.2396	0.0934	0.0512	23.56	21.37	43.19
0.2951	0.2331	0.2570	0.0978	0.0535	22.95	20.82	42.07
0.3089	0.2440	0.2690	0.1016	0.0555	22.74	20.63	41.68
Galacturonic acid B.							
0.2219	0.1835	0.2022	0.0789	0.0437	23.80	21.59	43.63
0.2771	0.2291	0.2525	0.0960	0.0525	22.92	20.79	42.01
Galacturonic acid C.							
0.1326	0.1067	0.1176	0.0426	0.0248	23.25	21.09	42.62
0.1803	0.1450	0.1599	0.0613	0.0346	23.82	21.61	43.66
Pectolic acid.							
0.1531	0.1488	0.1596	0.0623	0.0352	24.27	22.02	44.49
0.1662	0.1572	0.1732	0.0683	0.0382	24.27	22.03	44.49
0.1828	0.1729	0.1905	0.0748	0.0415	24.00	21.78	43.99
0.1864	0.1763	0.1943	0.0750	0.0416	23.59	21.40	43.24
0.2366	0.2237	0.2466	0.0944	0.0517	23.10	20.97	42.34
0.2407	0.2276	0.2509	0.0988	0.0540	23.72	21.52	43.48
Euxanthic acid.							
0.3938	0.1643	0.1811	0.0652	0.0365	22.21	20.15	40.71
0.4017	0.1676	0.1848	0.0628	0.0353	21.05	19.10	38.58
0.4049	0.1690	0.1863	0.0678	0.0379	22.42	20.34	41.10
0.4071	0.1699	0.1873	0.0667	0.0374	21.98	19.94	40.29
0.4146	0.1730	0.1907	0.0689	0.0385	22.22	20.16	40.73
0.4402	0.1837	0.2025	0.0680	0.0380	20.69	18.77	37.92
0.4416	0.1843	0.2031	0.0692	0.0386	20.95	19.01	38.40
0.4472	0.1866	0.2057	0.0677	0.0379	20.28	18.40	37.17

In this manner the yield of furfuraldehyde from each of the reference substances previously described was determined. In order to avoid any complications, the dry weight of the precipitated phloroglucide was taken and read as furfuraldehyde by means of Kröber's tables. For reasons which will be discussed in a later paper, the practice of washing the phloroglucide with hot alcohol adopted in many laboratories appears to be a somewhat questionable procedure, and the use of Kröber's tables for the alcohol-washed precipitate undoubtedly introduces errors. In the present instance, the weight of the precipitate after alcohol treatment was determined but is not recorded.

Table II shows the results of the furfuraldehyde determinations in full and yields of furfuraldehyde are shown as percentages of the uronic anhydride and of uronic acid present in the sample and compared with the theoretical.

In Table III the ratios of uronic acid and anhydride to phloroglucide and furfuraldehyde produced are shown, calculated from the averages of the results recorded in the first two tables.

Table III. *Average ratios of uronic compounds to phloroglucide and furfuraldehyde produced.*

Substance	Ratio of uronic anhydride to		Ratio of uronic acid to	
	Phloroglucide	Furfuraldehyde	Phloroglucide	Furfuraldehyde
Galacturonic acid A	2.38	4.30	2.62	4.75
Galacturonic acid B	2.36	4.29	2.60	4.72
Galacturonic acid C	2.43	4.25	2.69	4.69
Pectolic acid	2.33	4.20	2.56	4.63
Euxanthic acid	2.61	4.66	2.88	5.14

DISCUSSION.

The determination of uronic acids by decarboxylation has already been discussed and calls for no further comment. The results of the determinations of furfuraldehyde as shown in Table II raise several interesting points which require comment. Grouping the three samples of galacturonic acid together, and considering, for example, the yield of furfuraldehyde as a percentage of the uronic anhydride present, the average value is 23.38 % and the mean error of the determination is approximately 2 %. This value, in the case of pectolic acid, is 23.83 %, with a similar mean error of experiment. There is no significant difference between the yields of furfuraldehyde from galacturonic acid as such and from the acid in molecular combination, as in pectolic acid. Examination of the figures for euxanthic acid reveals a significant difference from the figures suggested above; in this case the average yield of furfuraldehyde from the glycuronic anhydride present is 21.48 %, with a mean experimental error of 3.8 %. The higher experimental error in this case is probably due to the presence of a preponderating amount of other organic material—euxanthone—in euxanthic acid, and this also may be responsible for the definitely lower yield of furfuraldehyde, compared with that from galacturonic compounds. From the available results, however, it must be concluded that glycuronic acid in organic combination yields a definitely smaller quantity of furfuraldehyde than does galacturonic acid similarly.

It has been indicated previously and we feel it advisable again to emphasise that the furfuraldehyde yields have been based solely on the application of Kröber's tables to the weight of furfuraldehyde phloroglucide obtained. The influence on the final result of such determinations, of variations in apparatus,

technique of distillation and after-treatment of the precipitate is now being studied. The results of this study which should shortly be available may necessitate a slight modification in the figures of Table IV which indicate the

Table IV.

	Galacturonic acid		Glycuronic acid	
	Present finding %	Nanji, Paton and Ling %	Present finding %	Lefevre and Tollens %
Yield of furfuraldehyde:				
Based on uronic anhydride	23.50	16.66	21.48	18.96
Based on uronic acid	21.33	15.11	19.36	17.21
As percentage of theoretical	43.10	30.54	39.37	34.76
Ratio of uronic anhydride to:				
Phloroglucide	2.36	3.32	2.61	2.93
Furfuraldehyde	4.26	6.00	4.66	5.28
Ratio of uronic acid to:				
Phloroglucide	2.60	3.66	2.88	3.23
Furfuraldehyde	4.69	6.61	5.14	5.83

final averages of all the results of the previous tables, and are compared with the corresponding values applied by Nanji, Paton and Ling to galacturonic acid and those found by Lefevre and Tollens in their study of euxanthic acid.

It will be seen that the proposed yields and factors differ considerably from those of Nanji, Paton and Ling and from those of Lefevre and Tollens. On the other hand, they agree very closely with the recent findings of Ehrlich, referred to previously. Ehrlich and co-workers suggest that the ratio of galacturonic acid to phloroglucide is 2.64 : 1, which agrees, well within the limits of experimental error, with our average ratio of 2.60 : 1.

Our higher yields of furfuraldehyde in comparison with those of Lefevre and Tollens would seem to indicate that under our conditions of distillation there is less destruction of furfuraldehyde, or furfuraldehyde-yielding substance. We have, in fact, accumulated some evidence that this is the case when dealing with pure pentoses and for this reason we have found Kröber's tables inapplicable to such sugars. This will be more fully discussed in the succeeding paper, since the present issue remains unaffected, as we have merely used Kröber's tables to deduce weight of furfuraldehyde from that of the phloroglucide.

The values given by Nanji, Paton and Ling are, of course, not experimental, but arose by some misconception of the work of Lefevre and Tollens. The former values are quoted, however, in order to indicate how far their uncritical acceptance has led many workers astray. The well-known six-membered ring formula for pectic acid proposed by Nanji, Paton and Ling and widely accepted in this country and to some extent elsewhere, was deduced by calculation from the erroneous factors. In view of the considerable difference between these and the more correct factors, it is unlikely that the ring formula is now tenable and revision is called for. The erroneous factors have since been employed in studies on pectin, hemicellulose, gums, mucilages, decomposition of straws, *etc.*, and in many cases results will require recalculation, and considerable revision of the original deductions may be necessary.

Some of the subjects mentioned above are already undergoing experimental revision in these laboratories; thus pectin and pectic acid are being re-examined

in the light of the more correct factors, and it is proposed to extend the observations to calcium pectate and its significance in the analysis of pectic substances.

In our view then, many of the experimental results in this particular field will require close scrutiny and the correction to be applied involves more than the mere substitution of the more correct furfuraldehyde/uronic acid factors. The temptation to follow this simple procedure was strong but was resisted since other factors are concerned. It is essential that we should know accurately the influence on the furfuraldehyde yield of pentoses and uronic acids, of hexoses and other organic substances likely to be present in cell wall material. This question has been studied to some extent and is dealt with in a recent paper by Peter *et al.* [1933] in which it is shown that hexoses have a marked influence on furfuraldehyde yield. Until we have many more data on the yield of furfuraldehyde from mixtures of the type commonly met with, it is idle to attempt recalculation. Further it should be stressed that the use of Kröber's tables, except for correlation of phloroglucide and furfuraldehyde, is quite unjustified, as the tables were constructed for pure xylose and arabinose, and not for mixtures containing only a proportion of these sugars.

It is hoped that the work now proceeding will provide at least some of the missing data and that the analysis of pectin and similar carbohydrates may be placed on a firmer basis.

SUMMARY.

1. The furfuraldehyde due to pentose material in cell wall substances may be determined by deduction from the total furfuraldehyde yield of that due to uronic acids.

2. The yields of furfuraldehyde phloroglucide and of furfuraldehyde from galacturonic, polygalacturonic and euxanthic acid are given, together with appropriate factors for calculation.

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