LXVII. THE DETERMINATION OF LIGNIN IN THE ANALYSIS OF WOODS.

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Of all quantitative determinations in the analysis of plant materials the lignin determination is subject to greatest uncertainty since in the first place the constitution of this component is unknown and secondly all attempts to isolate it in the form in which it is believed to occur in the plant have, so far, failed. At the present time any so-called quantitative determination of lignin is at best a compromise, for the yield and composition of the product depend to a great extent on the analytical method employed. The best available methods for the routine determination of lignin in wood are those which involve the removal of the carbohydrates by hydrolysis with mineral acids. The details of the original methods and various modifications of them which have appeared from time to time are fully discussed by Schorger [1926, p. 518] and Hawley and Wise [1926, p. 163].

A number of modifications of the original methods [Ost and Wilkening, 1910; Willstätter and Zechmeister, 1913] have been suggested. Most of these aim at the preparation of a lignin complex, free from carbohydrate condensation products, and broadly speaking they involve either (1) preliminary treatment to remove those carbohydrates which are liable to form condensation products at some stage in the isolation of the lignin residue [Paloheimo, 1925; Ritter *et al.*, 1932; Norman and Jenkins, 1934], or (2) control of experimental conditions, such as the temperature of the wood samples during digestion with concentrated acid [Sherrard and Harris, 1932; Peterson *et al.*, 1932; Ritter *et al.*, 1932; Komarov, 1934]. Cohen and Dadswell [1931] have shown that with certain Australian woods the accurate determination of lignin is further complicated by the presence of gums or kinos which are resistant to concentrated mineral acids. These substances are, however, soluble in dilute sodium hydroxide, and the above authors have therefore proposed a modification of the Ost and Wilkening method which involves a preliminary treatment with this reagent. A further modification has since been suggested by Cohen [1934].

Since the crux of the lignin determination lies in the efficient removal of carbohydrates, it is essential that uniformity of method should be established for the digestion with concentrated acid and the subsequent hydrolysis with dilute acid. Although the method of Willstätter and Zechmeister [1913], which prescribes the use of 42 % hydrochloric acid, has hitherto been considered to yield the most accurate results, considerations of convenience have led to the almost universal use of 72 % sulphuric acid, as originally recommended by Ost and Wilkening [1910]. Further, it has been indicated by Harris *et al.* [1934] that the sulphuric acid method is capable of yielding a lignin with maximum methoxyl content and free from carbohydrates. Billington *et al.* [1933], conclude that the modified sulphuric acid method of Ritter *et al.* [1932] yields results which are

almost as satisfactory as those obtained by the hydrochloric acid method. None the less, although the use of sulphuric acid is generally favoured, there is a complete absence of agreement concerning the significant steps in the lignin determination. For instance, although all the above workers use 2 g. samples of wood flour Peterson *et al.* digest with 30 ml. of 72 % H_2SO_4 at 4° for 18 hours, Sherrard and Harris recommend 20 ml. of 70 % H_2SO_4 at 10° for 16 hours, Ritter *et al.* recommend 25 ml. of 72 % H_2SO_4 at 20° for 2 hours and Komarov digests his wood samples for 2½ hours at 20–21° in the case of softwoods and 25–26° in the case of hardwoods.

The method which has been in current use in this laboratory is substantially that recommended by Schorger [1926, p. 524] except that 12.5 ml. of 72 % sulphuric acid have been used per 2 g. (air-dry) sample of wood, and the hydrolysis with 3 % acid has been carried out under a reflux condenser. The following work was undertaken with a view to revising this method, and it has necessarily involved the assessment of the relative merits of several of the methods referred to above. For the present, the question of the eradication of gums and kinos has been left aside.

EXPERIMENTAL.

The effect of a preliminary hydrolysis with dilute sulphuric acid on the yield of apparent lignin.

Norman and Jenkins [1933] have shown that in the case of certain straws the yield of apparent lignin can be substantially reduced by a pre-treatment with 5 % sulphuric acid. They also record small losses in the apparent lignin of Scots pine and beech wood due to the same treatment. However, as mentioned in the Forest Products Research Board Report [1933], experiments carried out in this laboratory indicate that this expedient is not applicable to all woods. The experimental data in Tables I and II support this view. In these and succeeding tables results are expressed as percentages by weight of oven-dry original 80–100 mesh wood.

Table I. *The effect of preliminary hydrolysis with 1% sulphuric acid for 5 hours at 100° on the apparent lignin content of wood.*

16 hours' digestion with 12.5 ml. 72 % H_2SO_4 at 15–20°; 2 hours' hydrolysis with 3 % acid.

Material	Apparent lignin content		Change in apparent lignin content
	Untreated sample	After preliminary hydrolysis with 1 % H_2SO_4	
African pencil cedar (<i>Juniperus procera</i> Hochst.)	37.10	40.77	+ 3.67
Louisiana Gulf cypress (<i>Taxodium distichum</i> Rich.):			
Heartwood (50 mesh)	31.82	32.08	+ 0.26
Sapwood	34.44	34.77	+ 0.33
Pitch pine (<i>Pinus palustris</i> Mill.):			
Highly resinous	21.18	21.43	+ 0.25
Slightly resinous	29.60	29.89	+ 0.29
English oak (<i>Quercus</i> sp.)	22.40	18.97	– 3.43
Jarrah (<i>Eucalyptus marginata</i> Sm.)	43.37	39.97	– 3.40
Teak (<i>Tectona grandis</i> L.f.)	30.53	31.76	+ 1.23
Abura (<i>Mitragyna stipulosa</i> Kuntze)	32.75	33.40	+ 0.65

In all cases, except oak and jarrah (Table I), increases in apparent lignin are observed. These increments probably arise from the formation of insoluble products from partially hydrolysed carbohydrates, although in the light of more recent knowledge, it is uncertain whether the cause of the increments is to be sought in the conditions of the preliminary hydrolysis alone.

Preliminary hydrolysis with 5% sulphuric acid.

Three of the woods used in Table I were selected for the determinations recorded in Table II, the preliminary hydrolysis being carried out on 2 g. (air-dry) samples of wood flour with 100 ml. 5% sulphuric acid at 100° for 3 hours. The wood residue was in each case collected in an alundum crucible (porosity RA 98) in the usual manner.

Table II. *The effect of preliminary hydrolysis with 5% sulphuric acid for 3 hours at 100° on the apparent lignin content of wood.*

Material	Apparent lignin content		
	Untreated sample	After hydrolysis with 5% H ₂ SO ₄ for 3 hours at 100°	Change in apparent lignin content
African pencil cedar	37.59	39.18	+ 1.59
Louisiana Gulf cypress	32.67	32.52	- 0.15
English oak: sapwood	23.10	20.31	- 2.79

The only results that can be directly compared with those of Norman and Jenkins [1934] are those for oak and teak. For oak wood, these authors observed a loss of 8.56 parts of apparent lignin and for teak a loss of 2.03 parts, after hydrolysing for 1 hour with 5% sulphuric acid at 100°. Since, however, of the 7 species examined here only two show a decrease in apparent lignin, it is obvious that the introduction of this type of preliminary hydrolysis as a refinement in the lignin determination is open to criticism.

Preliminary hydrolysis with dilute sulphuric acid with immediate removal of the products of hydrolysis.

A more successful pre-treatment was suggested by the work of Paloheimo [1929] who carried out two successive digestions with 70% sulphuric acid. The concentration of hydrolysis products during the second digestion was thus considerably reduced, and for rye straw a lower yield of lignin was obtained.

In the following series of experiments (Table III) 2 g. samples of air-dry wood in alundum crucibles of porosity RA 98 were hydrolysed either before or after extraction with alcohol-benzene with boiling dilute sulphuric acid in a series of stages. 25 ml. of acid were added at each stage and removed by suction along with the hydrolysis products. After the final stage the samples were washed free from acid with hot water before carrying out the usual lignin determination.

These results show that it is possible by using 5% acid to reduce the apparent lignin content of beech and African pencil cedar by over 1% without appreciable loss of methoxyl. 10% sulphuric acid appears to attack the lignin of beech wood since the methoxyl is depleted.

Table IV shows the distribution of pentosans after treatment (3). The loss of 1% apparent lignin in the case of cedar is apparently not due to the removal of pentosans. On boiling the acid extract for 2 hours, however, an insoluble residue resembling lignin was obtained. This amounted to 0.35% in

Table III. *The effect of a preliminary hydrolysis with removal of hydrolysis products on the apparent lignin contents of beech and African pencil cedar.*

Treatment	African pencil cedar			Beech		
	Lignin	Methoxyl in lignin (i)	(i) as % of lignin	Lignin	Methoxyl in lignin (ii)	(ii) as % of lignin
1. Original wood	37.73	4.95	13.12	23.53	4.79	20.35
2. Alcohol-benzene extraction followed by treatment with 250 ml. hot 1% H ₂ SO ₄ for 2-min. periods	37.96	5.00	13.18	24.49*	4.71	19.25
3. Alcohol-benzene extraction followed by treatment with 250 ml. hot 5% H ₂ SO ₄ for 2-min. periods	36.72	4.91	13.36	22.00	4.52	20.55
4. Alcohol-benzene extraction followed by treatment with 500 ml. hot 5% H ₂ SO ₄ for 2-min. periods	37.56†	5.03	13.41	23.36†	4.50	19.27
5. Treatment with 250 ml. hot 5% H ₂ SO ₄ for 2-min. periods prior to alcohol-benzene extraction	36.63	4.78	13.05	22.71	4.59	20.22
6. Treatment with 250 ml. hot 5% H ₂ SO ₄ for 4-min. periods prior to alcohol-benzene extraction	36.54	4.77	13.06	22.45	4.63	20.63
7. Treatment with 250 ml. hot 10% H ₂ SO ₄ for 2-min. periods prior to alcohol-benzene extraction	37.19	4.93	13.25	22.02	4.26	19.83

* Temperature of digestion with 72% H₂SO₄, 25.5–29°.† Temperature of digestion with 72% H₂SO₄, 24.5°.Table IV. *Distribution of pentosans after treatment.*

	African pencil cedar	Beech
Total pentosans in original wood	11.10	25.59
Pentosans in residual wood after treatment (3)	10.85	23.47
Pentosans in 5% H ₂ SO ₄ extract	Nil	1.12
Insoluble residue from 5% H ₂ SO ₄ extract	0.35	0.11
5% sulphuric acid extract	6.55	2.56

the case of cedar, and it would undoubtedly have been estimated as lignin had not precautions been taken to remove the products of hydrolysis as they were formed (*cf.* Table II).

Unfortunately in the experiments in which 500 ml. of 5% sulphuric acid were used the digestions with 72% acid were carried out at a room temperature of 24.5°, some 5–8° higher than in the other experiments. This made the results useless for their original purpose, since the unexpectedly high values obtained for apparent lignin were in all probability due to the effect of increased temperature. Before continuing with the investigation of hydrolytic pre-treatments therefore a series of determinations was carried out to determine the maximum increase in apparent lignin that might be caused by summer temperatures in this country.

The influence of summer temperatures on the lignin determination.

Lignin determinations were made on a number of woods according to the method which has been in current use in this laboratory, except that the digestion with 72 % sulphuric acid was carried out in an oven maintained at 33°, the highest laboratory temperature likely to occur during summer in England. Table V shows these results compared with those obtained with the same woods at temperatures of 15–20°.

Table V. *Effect of temperature of digestion with 72% sulphuric acid on apparent lignin content.*

Temperature during digestion with 72% H ₂ SO ₄	15–20°			33°			Increase in apparent lignin due to temperature
	Apparent lignin	Methoxyl in lignin (i)	(i) as % of lignin	Apparent lignin	Methoxyl in lignin (ii)	(ii) as % of lignin	
African pencil cedar	37.73	4.95	13.12	40.00	4.98	12.44	2.27
Louisiana Gulf cypress	32.67	5.21	15.95	35.20	5.20	14.77	2.53
Sitka spruce (<i>Picea sitchensis</i> Carr.)	27.83	4.14	14.88	29.74	4.23	14.23	1.91
Pitch pine, slightly resinous	29.19	4.27	14.63	31.15	3.70	11.89	1.96
Norway spruce (<i>Picea Abies</i> Karst)	26.05	4.10	15.73	27.95	4.04	14.45	1.90
Beech (<i>Fagus sylvatica</i> Linn.)	23.53	4.79	20.35	27.98	4.95	17.70	4.45
Ash (<i>Fraxinus excelsior</i> Linn.)	24.75	4.92	19.88	28.88	5.05	17.48	4.13
English oak	22.40	3.90	17.44	27.43	3.86	14.03	5.03
Jarrah	43.37	5.84	13.48	47.94	5.99	12.48	4.57

It can be seen that a relatively high temperature affects the yield of hardwood lignin much more than that of softwood lignin. This may be accounted for by the greater proportion of readily hydrolysable carbohydrates present in hardwoods. These results amply confirm the observations of Sherrard and Harris [1932] and Peterson *et al.* [1932].

The optimum conditions for carrying out the determination of lignin with 72% sulphuric acid.

Ritter *et al.* [1932] have published graphs showing the effect of temperature on the yield of lignin from the wood of sugar maple after digestion with 72 % sulphuric acid for periods ranging from 1 to 96 hours. They conclude that a digestion for 2 hours at 20° ensures complete solution of the carbohydrates with a minimum amount of decomposition and adopt this for future use, although it would appear from their graphs that a minimum yield of lignin is actually obtained by digesting for 7 hours at 10°. Consideration of these graphs and the results of other workers [Sherrard and Harris, 1932; Peterson *et al.*, 1932] has led us to adopt 10° as the best temperature for the digestion.

The authors desire to record here their indebtedness to Mr J. Piqué of the Low Temperature Research Station, Cambridge, for supplying the design of a constant temperature chamber in which it has been possible to maintain a

temperature of $10 \pm 0.5^\circ$ for prolonged periods. Full details of the complete apparatus can be obtained on request from the Director of Forest Products Research.

The effect of crucible porosity on the yield of apparent lignin from certain hardwoods.

A set of determinations was carried out on beech, ash and English oak to compare the efficiency of alundum crucibles of porosity RA 98 and 360 respectively in retaining the lignin precipitate (Table VI).

Table VI. *The effect of crucible porosity—RA 98 compared with RA 360.*

16 hours' digestion with 72% H_2SO_4 at 10° ; 2 hours' hydrolysis with 3% acid.

Volume of 72% H_2SO_4 used ml.	Beech				Oak, sapwood				Ash			
	Apparent lignin		Methoxyl in lignin		Apparent lignin		Methoxyl in lignin		Apparent lignin		Methoxyl in lignin	
	RA 98	360	98	360	98	360	98	360	98	360	98	360
12.5	22.36	22.81	4.61	4.63	19.92	20.21	4.17	4.18	23.24	23.57	4.75	4.78
20	22.25	22.60	4.65	4.69	19.34	19.54	4.13	4.16	22.54	23.17	4.69	4.72
25	22.28	22.30	4.58	4.67	19.10	19.25	4.02	4.13	22.43	23.00	4.70	4.78
30	21.82	22.10	4.48	4.63	18.86	19.44	4.00	4.13	22.27	22.87	4.67	4.77

In all cases a slightly higher yield of apparent lignin was obtained by using crucibles of porosity RA 360 and the methoxyl content of the lignin collected in these was constant irrespective of the volume of sulphuric acid used. Crucibles of porosity RA 360 have therefore been adopted for collecting lignin in all future work.

The influence of the volume of 72% sulphuric acid used on the yield of apparent lignin.

2 g. samples of air-dry, alcohol-benzene (1:2)-extracted sawdust were digested with different volumes of 72% sulphuric acid at 10° for 16 hours. The acid was then diluted to a concentration of 3% and the mixture boiled for 2 hours before collecting the lignin residue (Table VII).

Table VII. *The effect of using different volumes of 72% sulphuric acid.*

Volume of 72% H_2SO_4 used ml.	Beech		African pencil cedar		English oak sapwood		Norway spruce		Ash		Douglas fir	
	Meth-		Meth-		Meth-		Meth-		Meth-		Meth-	
	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin
12.5	22.81	4.63	38.06	4.94	20.21	4.18	25.63	4.15	23.57	4.78	26.80	4.02
20	22.60	4.69	37.81	5.07	19.54	4.16	25.37	4.09	23.17	4.72	26.55	3.92
25	22.30	4.67	37.33	4.95	19.25	4.13	25.42	4.07	23.00	4.78	26.54	3.99
30	22.10	4.63	37.45	4.94	19.44	4.13	25.33	4.08	22.87	4.77	26.44	4.01

Consideration of the data suggests that for a 2 g. sample of wood containing approximately 10% of moisture 25 ml. of 72% sulphuric acid be adopted.

The effect of varying the time of contact of wood samples with 72% sulphuric acid on the yield of apparent lignin.

Table VIII gives the results obtained by digesting 2 g. samples of various woods with 25 ml. of 72% sulphuric acid at 10° for periods of 3 to 16 hours, the acid being subsequently diluted to 3% and the mixture boiled for 2 hours before collecting and washing the lignin in the usual way.

Table VIII. *The effect of varying the time of contact of wood with 25 ml. 72% sulphuric acid.*

Time of digestion with 72% H ₂ SO ₄ at 10° (hours)	Beech		Cedar		English oak, sapwood		Norway spruce		Ash		Douglas fir	
	Meth-		Meth-		Meth-		Meth-		Meth-		Meth-	
	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin
3	34.58	4.70	42.83	5.58	25.55	4.07	30.88	4.56	30.96	4.81	34.05	4.23
4	21.60	4.46	42.02	5.44	19.00	3.94	25.58	4.14	23.12	4.69	30.43	4.31
5	21.60	4.45	37.31	4.90	18.47	3.92	25.34	4.05	22.50	4.69	26.77	3.96
6	21.62	4.49	37.27	4.99	19.03	4.05	25.14	4.04	22.75	4.65	26.61	3.87
7	21.70	4.47	37.23	5.01	18.86	4.05	25.40	4.06	22.67	4.76	26.70	3.82
16	22.30	4.67	37.33	4.95	19.25	4.13	25.42	4.07	23.00	4.78	26.54	3.99

The data suggest that for the woods examined hardwoods require 5 hours' digestion with 72 % sulphuric acid, whilst softwoods require 6 hours' digestion.

The effect of varying the time of hydrolysis with 3% sulphuric acid on the yield of apparent lignin.

The conditions of the final hydrolysis with dilute acid have perhaps been subject to fewer modifications than any other part of the lignin determination. Nevertheless Ritter *et al.* [1932] advocate an extension of the time of hydrolysis at the boiling-point of the acid from the usual 2 to 4 hours, whilst Komarov [1934] appears to consider that 1 hour's hydrolysis with acid of 5 % concentration is sufficient.

After extraction with alcohol-benzene (1:2) 2 g. samples of air-dry wood were digested with 25 ml. of 72 % sulphuric acid at 10° for 5 hours (Table IX(a)) and 6 hours (Table IX(b)). The acid was diluted to 3 % and the final hydrolysis at the boiling-point was carried out for various times.

Table IX. *The effect of varying time of hydrolysis with 3% sulphuric acid on the yield of apparent lignin, using:*

(a) 25 ml. 72% H₂SO₄ for 5 hours at 10°.

Time of hydrolysis with 3% acid (hours)	Beech		Cedar		English oak, sapwood		Norway spruce		Ash		Douglas fir	
	Meth-		Meth-		Meth-		Meth-		Meth-		Meth-	
	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin	Appar-ent lignin	oxyl in lignin
1	21.62	4.47	37.37	5.06	18.61	3.94	25.73	4.17	22.44	4.70	26.77	4.01
2	21.60	4.45	37.31	4.90	18.47	3.92	25.34	4.05	22.50	4.69	26.77	3.96
3	21.64	4.51	37.75	5.04	18.49	3.95	25.50	4.19	22.23	4.70	26.50	4.01
4	21.64	4.59	37.86	4.99	18.51	4.03	25.48	4.12	22.20	4.70	26.67	3.99

(b) 25 ml. 72% H₂SO₄ for 6 hours at 10°.

Time of hydrolysis with 3% acid (hours)	African pencil cedar		Norway spruce		Douglas fir	
	Methoxyl in lignin		Methoxyl in lignin		Methoxyl in lignin	
	Apparent lignin	Methoxyl in lignin	Apparent lignin	Methoxyl in lignin	Apparent lignin	Methoxyl in lignin
1	37.89	4.99	25.36	4.07	26.83	3.92
2	37.27	4.99	25.14	4.04	26.61	3.87
3	37.53	4.98	25.14	4.11	26.65	3.94
4	37.49	4.93	25.36	4.17	26.65	3.98

These results indicate that with the exception of ash wood 4 hours' hydrolysis with 3 % sulphuric acid is unnecessary for the woods examined. Moreover, in particular cases, the longer hydrolysis may lead to high values for lignin. From a practical standpoint it is obviously important that the time taken for the complete lignin estimation should be reduced to a minimum and therefore 2 hours is proposed as the time for the hydrolysis with 3 % sulphuric acid.

The action of concentrated sulphuric acid on sugars as a source of error in the lignin determination.

Hilpert and Littmann [1934] have confirmed and amplified the earlier work of Paloheimo [1929] on the action of concentrated mineral acids on sugars in relation to the determination of lignin and have shown that whereas glucose yields 0.81 % of an insoluble lignin-like product when treated with 72 % sulphuric acid for 48 hours at 20–22°, fructose may yield 25 %, sucrose 13–14 % and xylose 36 %. This so-called resinification of sugars is regarded as an important source of error in the lignin determination in plant materials generally, but in a later paper [1935] it is shown that the reaction is profoundly affected by changes in temperature. At 6° fructose and xylose only yield 0.26 and 0.01 % respectively of an insoluble lignin-like residue.

In the following experiments 1 g. samples of pure xylose, fructose and sucrose respectively were mixed with 25 ml. 72 % sulphuric acid at 10° and maintained at that temperature for 5 hours. Any insoluble precipitate formed was collected in an alundum crucible of porosity RA 360, washed with water and dried at 105° (Table X).

Table X. *Effect of 72% sulphuric acid on sugars at 10°.*

	Insoluble residue (%)
Xylose	0.07
Sucrose	0.04
Fructose	0.01

It may be concluded that the possible error introduced into the determination of lignin in wood by the presence therein of small quantities of sugars can hardly be greater than the experimental error of the determination, provided that the digestion with 72 % H_2SO_4 is carried out at 10°.

DISCUSSION.

The experimental work described above will have served a useful purpose if it only succeeds in focussing attention on the wide variations in technique at present being employed in different laboratories in the estimation of lignin by means of sulphuric acid. From a consideration of all the available data on this subject, there can be no doubt that so long as the majority of workers continue to estimate lignin by the same general method the conditions of the digestion with concentrated acid must be standardised. Reasons have been given for the adoption of 10° as the most suitable temperature for this digestion and of 25 ml. as the most suitable volume of 72 % sulphuric acid. The question of the most appropriate time of digestion may not be so easily settled, but the data show that it can be shorter for hardwoods than for softwoods.

The preparation of the wood sample for the main hydrolysis is the step in the determination which will from time to time be subject to the greatest

modifications. Chemically the greatest variants in wood substance are the minor components and it is not surprising that as more species of wood are examined it is being found that the minor components cannot always be effectively removed by the usual neutral solvents. This has become particularly apparent in certain of the Australian hardwoods examined by Cohen and his co-workers [1931; 1934].

It is highly probable that the removal of minor components prior to the lignin determination proper will ultimately constitute a different problem for different types of wood, but further discussion of this aspect of the problem would appear to be unprofitable at present. Meantime thorough extraction with alcohol-benzene (1:2) followed by washing with hot water is recommended for woods which are not conspicuously high in extractives in the belief that further work on the extractive problem can best be interpreted after the main steps in the lignin determination proper have been settled.

Greater accuracy may conceivably be introduced into the lignin determination by applying a preliminary hydrolysis with dilute acid, provided that the products of hydrolysis are removed as soon as possible. In this connection the results obtained in the earlier part of this investigation are encouraging and the matter will be followed up as time permits.

SUMMARY.

1. Current methods for determining lignin in wood by means of 72 % sulphuric acid are briefly reviewed and the present lack of uniformity in these methods is stressed.

2. Preliminary hydrolysis of the wood sample with dilute sulphuric acid has been shown to be ineffective as a general means of preventing the formation of carbohydrate condensation products during the isolation of lignin, unless precautions are taken to remove the products of hydrolysis as soon as possible after they are formed.

3. The conditions governing the digestion of the wood sample with 72 % sulphuric acid have been studied in detail and on the basis of the data obtained the following procedure is suggested for the determination of lignin in wood. After a preliminary extraction with alcohol-benzene (1:2), a 2 g. sample of air-dry wood of approximately 10 % moisture content is digested with 25 ml. of 72 % sulphuric acid at $10^{\circ} \pm 0.5^{\circ}$ for 5 hours in the case of hardwoods and 6 hours in the case of softwoods. The acid is diluted with water to a concentration of 3 % and the mixture boiled under reflux for 2 hours. The lignin residue is then collected in an alundum crucible of porosity RA 360, washed till free from acid and dried at 105° .

4. It has been shown that when xylose, fructose and sucrose are treated with sulphuric acid under the conditions stated above only negligible amounts of insoluble residues are obtained.

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