LIGNIN STUDIES

II. *The lignin content and properties of lignin in different materials*

MAIJA-LIISA SALO

Department of Animal Nutrition and Husbandry, University of Helsinki

Received Juni 15, 1957.

Referring to the lack of a color reaction with phloroglucinol and hydrochloric acid GJOKIC (ref. BRAUNS, 3), at the end of the last century, concluded that lichens and mosses do not contain lignin. LINSBAUER (ibid.) confirmed Gjokic's results and found also the fungi lacking in lignin, this being first observed in the group of *Phanerogamae* and *Pteridophyta*. According to KRATZL and EIBL (9) humuslike substances are obtained by the acid methods also from the plants of the group of *Bryophyta*, but these plants give no color reactions of lignin and on oxidation no vanillin is formed. LINDBERG and THEANDER (11), on the other hand, mention that some *Sphagnum* species contain about 5 % lignin. This lignin was, however, exceptional in the way that its methoxyl content was only 1 % and the aldehydes obtained on oxidation contained 90 % p-hydroxybenzaldehyde, a substance which is obtained only in traces from the lignin of the group of *Phanerogamae*. HOLMBERG (7), who determined lignin as lignothioglycolic acid, found that the composition of lignothioglycolic acids obtained from the leaf stems of *Pteris aquilina* and from the stems of *Lycopodium annotinum* were identical with the corresponding substance from spruce wood, whereas the lignothioglycolic acid from lichens, mosses, and fungi had a different composition.

The lignin content of different woods has been the subject of several investigations (8, 3). Also the lignin percentages of various grasses and clovers may be found in numerous papers (17, 18, 16,1). However, information about other materials is very scanty.

Experimental methods and results

In this investigation the content of lignin, methoxyl in lignin, the total of membrane substances, and crude protein were studied in different materials. The lignin was determined by the method (method A) developed in this laboratory and described in a previous paper (20). The amount of membrane substances was determined by the method of PALOHEIMO (15) and the methoxyl content of lignin by a modification of ZEISEL method (13).

The results are given in Table 1. The materials are grouped in the first place according to their botanical association. The upper part on the Table refers to the changes occurring in the chemical composition of grasses and red clover at successive stages of growth. The series of figures in all columns are characteristic of the maturing of the plants. The percentage of crude protein decreases whereas the content of membrane substances, of lignin, of lignin in membrane substances, and of methoxyl in lignin, increases. The figures obtained from rye and timothy are in agreement. Also the oat hulls are typical gramineae material: e.g. the lignin content of cell walls and the methoxyl content of lignin agree with the corresponding values of mature rye and timothy. Red clover is somewhat poorer in membrane substances than the grasses, however, the cell walls are somewhat more lignified in clover than in rye and timothy. Some of the analytical data belonging to the wheat bran are rather similar to the corresponding figures concerning young red clover and the leaves of clovers. As to wheat bran, however, the method for the determination of the total of membrane substances gives too low a value (15), a fact that explains the high percentage of lignin in membrane substances in this material.

In the samples of wood the percentages of membrane substances are very similar but in softwoods the lignin content is higher than in hardwoods and they contain less methoxyl. The chemical composition of peanut hulls, prune stone shells and leaf stems of *Eupteris aquilina* agrees with the composition of softwoods. Peanut hulls resemble softwoods even in that respect that their lignin does not dissolve in hot diluted alkali solution. The prehydrolysis with diluted acid removes from hardwoods substances having a methoxyl content very similar to that of lignin. For that reason the lignin percentages presented by different investigators are rather variable, while the discrepancies in the methoxyl content of the lignin are small (8, 3). Some wood chemists (2, 4) reject the use of prehydrolysis in the lignin determination of woods.

The lignin persentages in cork and pine bark are very high and the methoxyl content of lignin is low. The lignin preparations may contain suberin.

The root materials contain very small amounts of membrane substances and the methoxyl content of lignin is high in comparison with the low lignin percentage.

The last group in the Table consists of plants which, according to general opinion, may not contain lignin. Larger or smaller quantities of residue is, however, obtained from all of them in the lignin determination, but the methoxyl content of this residue is very low. The high lignin percentage of *Polytricum commune* corresponds with the fact that the hard central cord of the stem does not dissolve in a lignin determination.

The figures in the last column of Table 1 show that the richer the material is in protein, and the poorer in membrane substances, the more the lignin preparation contains nitrogenous impurities.

The fifth column of the Table indicates that the methoxyl content of lignin varies in different materials. However, methoxyl is considered one of the most important analytical criteria of lignin (3). According to FREUDENBERG et al. (6) in lignin methoxyl must be attached to an aromatic ring in an ether form. On

Table 1. Some analytical data from different plant materials

Material	Crude protein % of dry matter	Membrane substances % of dry matter	Lignin % of dry matter	Lignin % of membrane substances	Methoxyl in lignin % of lignin	Crude protein in crude lignin % of crude lignin
Rye, early leaf stage	16.4	22.4	2.1	9.4	5.6	33.3
» early head stage	5.8	50.5	5.3	10.5	13.7	11.0
» in bloom	7.8	64.7	8.3	12.8	15.6	12.5
» straw	1.7	82.2	11.7	14.2	15.7	3.0
Timothy, leaf stage	15.7	37.2	3.1	8.3	9.5	26.3
» early head stage	8.7	43.4	4.1	9.4	10.8	19.9
» in bloom	7.8	61.1	8.0	13.1	12.9	12.6
» straw	2.5	62.6	9.4	15.0	16.0	5.1
Red clover, leaf stage	26.4	16.6	2.7	16.3	4.9	41.0
» in bud	18.9	30.1	4.8	15.9	9.4	32.3
» in bloom	14.6	38.2	6.6	17.3	11.1	26.3
» after blooming	12.2	52.4	10.5	20.0	12.5	20.8
» leaves [1]	33.2	16.1	3.4	21.1	4.0	51.7
» upper part of the stalks [1]	14.5	38.2	5.6	14.7	9.2	20.0
» lower » » » » [1]	8.7	55.1	8.1	14.7	14.6	12.2
Alsike clover, leaves [1]	30.2	15.3	3.6	23.5	4.0	48.0
» stalks [1]	9.9	43.6	6.3	14.4	13.1	15.8
Spuce	0.5	93.0	23.0	24.7	15.6	0.5
Pine	0.4	90.1	25.7	28.5	15.3	0.5
Birch	0.6	91.6	12.7	13.9	21.6	1.3
Oak	0.9	85.7	16.2	18.9	20.2	1.7
Pine bark	1.3	78.8	57.6	73.1	4.0	1.0
Cork	3.0	82.9	69.9	84.3	5.7	2.2

Wheat bran	18.3	17.0	4.7	27.6	4.3	15.9
Oat hulls	1.4	72.5	10.6	14.6	15.2	2.7
Prune stone shells	1.2	89.2	23.6	26.5	19.0	1.3
Peanut hulls	5.9	82.6	27.5	33.3	14.6	6.9
Sugar beet pulp	11.4	42.8	2.1	4.9	10.2	33.3
Rutabaga, rind	16.2	17.0	1.6	9.4	10.9	31.5
» pulp	9.7	11.1	0.5	4.5	6.0	33.6
Eupteris aquilina, leaf stems	2.4	74.2	21.5	29.1	13.5	3.1
Equisetum pratense	25.6	23.9	2.5	10.5	2.1	36.5
Polytrichum commune	6.6	68.0	20.3	29.9	0.3	8.2
Sphagnum recurvum	6.5	68.3	2.2	3.2	1.2	29.8
Cladophora glomerata	10.7	22.2	6.1	27.5	0.3	12.5
Cladonia alpestris	3.8	68.4	0.8	1.1	1.2	20.7

[1]) At the beginning of blooming.

oxidation three aldehydes are obtained from lignin and these aldehydes differ only in regard to the number of methoxyl groups: syringaldehyde has two methoxyl groups, vanillin one, and p-hydroxybenzaldehyde none (5, 19, 10). As can be seen from the Table, in grasses and in red clover the methoxyl percentage of lignin increases when the plants mature. The determinations made from different parts of red clover show that the influentical factor is not directly the age of the plant but the quality of the tissue. With regard to the methoxyl content in lignin, the lignins in red clover at the leaf stage and in the leaves of older clovers are similar. In the upper and lower parts of the stalks of red clover the methoxyl content of lignin is rather different. The same is to be seen in the rind and in the pulp of rutabaga. When the materials presented in Table 1 are grouped on the basis of the methoxyl content of lignin the following groups can be formed.

a) 2 % or less methoxyl in lignin: mosses, sea-weed, lichen and horsetail.

b) 4—6 % methoxyl in lignin: grasses, and red clover at the early stage of development, the leaves of clovers, wheat bran, pulp of rutabaga, pine bark and cork.

c) 14—16 % methoxyl in lignin: the straw of Gramineae plants, the lower part of the stalks of red clover at blooming time, softwoods, peanut hulls, and the leaf stems of fern.

d) 19—21 % methoxyl in lignin: prune stone shells, and hardwoods.

This indicates that the methoxyl content of lignin varies within wide limits. It can be considered as a criteria of the purity of lignin only in comparing the lignin preparations isolated by different methods from identical material, and even then with certain reservations. It is possible that in the treatments non- lignin methoxyl is left in the preparations or that a part of the lignin methoxyl dissolves.

In order to study the solubility of lignin in hot diluted alkali the preparations were, after the usual prehydrolysis in 2 N acid, boiled in alkali solutions of varying concentrations. As can be seen from Table 2, about 50 % of the lignin of hay and of rye straw dissolves in an 0.1 N boiling natrium hydroxide solution, the corresponding figure for cow faeces being 57.7. However, of the lignin of softwoods even in 2 N alkali only 2—6 % dissolves. The difference may be caused by the structural diversity of the lignin or by the fact that lignin in various materials differs in its physical or chemical association with other cell wall components.

Even the usual lignin determination reveals a difference in the behaviour of lignin in different plant materials. When the prehydrolysis residue is treated with 72 per cent sulphuric acid or with 41 per cent hydrochloric acid and the strong acid is diluted with water, a flocculent precipitate is formed. Thus the lignin preparations isolated by the usual acid methods contain, besides the real hydrolysis residue, also substances dissolved by the strong acid but precipitated by the succeeding dilution. This fact was recognised by PALOHEIMO (14) and confirmed by MOON and ABOU-RAYA (12), who all considered also the precipitated fraction as lignin. Moon and Abou-Raya refer to their observation that the precipitate contains methoxyl and gives the color reaction of lignin.

The author's experiments revealed that the relation between insoluble and precipitated lignin fractions varies with the material. Further it varies with the

Table 2. The solubility of lignin in hot dilute alkali solutions.

Material [1]	Normal method Lignin % of dry matter	Method completed with boiling in alkali solution							
		0.01 N NaOH-solution		0.1 N NaOH-solution		0.25 N NaOH-solution		2 N NaOH-solution	
		Lignin % of dry matter	Dissolved from lignin, %	Lignin % of dry matter	Dissolved from lignin, %	Lignin % of dry matter	Dissolved from lignin, %	Lignin % of dry matter	Dissolved from lignin, %
Spruce	23.8	—	—	23.2	2.6	23.2	2.6	23.4	1.7
Pine	23.5	—	—	22.3	5.1	22.4	4.7	22.0	6.4
Oak	15.4	14.6	5.2	11.8	23.4	10.5	31.8	9.8	36.4
Rye straw	11.7	10.8	7.7	5.8	50.4	3.6	69.2	3.8	67.5
Hay [2]	8.1	6.9	14.8	4.2	48.1	3.7	54.3	2.9	64.2
Cow faeces	16.3	13.7	16.0	6.9	57.7	5.4	66.9	4.3	73.6

[1]) All of the samples are not identical with those in Table 1.

[2]) Timothy with some red clover.

Table 3. Fraction of lignin dissolved by 72 per cent sulphuric acid and precipitated by the succeeding dilution.

Material [1])	Lignin % of dry matter	Precipitated fraction (N-free) % of dry matter	% of lignin	Methoxyl content of lignin %	of the N-free precipitated fraction, %	Crude protein content of crude lignin %	of precipitated crude lignin %
Spruce	23.0	0.4	1.7	15.5	11.2	0.5	0.0
Pine	23.5	0.7	3.0	—	—	1.3	1.4
Birch	10.9	2.6	23.9	21.6	26.7	1.4	2.2
Oak	15.4	3.3	21.4	—	—	1.3	1.2
Rye straw	11.7	1.9	16.2	15.7	17.0	5.3	5.9
Hay	8.1	1.2	14.8	—	—	16.5	16.7
Cow faeces	15.4	1.4	9.1	9.5	12.5	22.5	28.4
Timothy, leaf stage	4.1	0.6	14.6	7.2	10.3	32.2	55.7
Red clover, »	4.0	1.3	32.5	4.2	2.1	47.0	53.8
Wheat bran	4.7	0.4	8.5	4.3	6.9	15.9	41.0

[1]) All the samples are not identical with those in Tables 1 or 2.

quality of the strong acid used in the lignin determination. If a 41 per cent hydrochloric acid is used, the precipitated fractions are less than a half of what they are with a 72 per cent sulphuric acid. Thus the precipitated fraction is hardly an inherent fraction of lignin. In Table 3 are given the amounts of precipitated lignin fractions, their methoxyl content, and the crude protein ($6.25 \times N$) content of the precipitated crude lignin. The results show that the methoxyl content of the precipitated fraction is generally very high, which points to lignin. On the other hand, plenty of nitrogenous substances precipitate by dilution so that the precipitated fraction of the crude lignin is still more impure than the lignin preparation as a whole.

Summary

In this investigation the lignin, the total of membrane substances, the crude protein and the methoxyl in lignin were determined in different materials. Some observations on the properties of lignin have been made.

The lignin content, as calculated from the dry matter or from the total of the membrane substances, varies greatly in different materials, as can be seen in Table 1. This is the case even if one leaves out of consideration such materials as the bark of woods, sea-weeds, and mosses, the »lignin» of which scarcely is real lignin. In grasses and clover the content of lignin in the cell walls increases with the successive stages of development.

The methoxyl content of lignin varies in different plants, in the same plant at successive stages of growth, and in the different tissues of the same plant.

The solubility of lignin in hot diluted alkali solutions varies in different materials. Of the lignin in Gramineae plants even in an 0.1 N natrium hydroxide solution about 50 % dissolves, but of the lignin of softwoods only negligible amounts in a 2 N solution.

The lignin preparations isolated by the usual acid methods contain, besides the real hydrolysis residue, also substances dissolved by the strong acid but precipitated by the succeeding dilution. The nitrogen content of the precipitated fraction is high. The high methoxyl content of its nitrogen free portion points to real lignin.

Acknowledgement. The author is indebted to Prof. Lauri Paloheimo for his valuable help in this work.

REFERENCES

(1) Armstrong, D. G. & Cook, H. & Thomas, B. 1950. The lignin and cellulose contents of certain grassland species at different stages of growth. J. Agric. Sci. 40: 93—99.

(2) Bamford, K. F. & Campbell, W. G. 1936. The determination of lignin in the analysis of woods. Biochem. J. 30: 419—428.

(3) Brauns, F. E. 1952. The chemistry of lignin. 808 p. New York.

(4) Cohen, W. E. & Harris, E. E. 1937. Pretreatment of wood with hot dilute acid. Effect on lignin values. Ind. Engin. Chem. Anal. Ed. 9: 234—235.

(5) Creighton, R. H. J. & Gibbs, R. D. & Hibbert, H. 1944. Studies on lignin and related compounds Am. J. Chem. Soc. 66: 32—37.
(6) Freudenberg, K. & Belz , W. & Niemann, C. 1929. Die aromatische Natur des Lignins. Ber. deut. chem. Ges. 62: 1554—1561.
(7) Holmberg, B. 1934. Thioglykolsäure als Ligninreagans. Ing. vetenskaps handl. no 131.
(8) Hägglund, E. 1951. Chemistry of wood. 631 p. New York.
(9) Kratz l, K. &. Eibl, J. 1951. Chemical and botanical evidence for lignification. Mitt. österr. Ges. Holzforsch. 3: 77. (Ref. Chem. Abstr. 46: 8366.)
(10) Leopold, B. & Malmström, I.-L. 1952. Studies on lignin. IV. Investigation on the nitrobenzene oxidation products of lignin from different woods by paper partition chromatography. Acta chem. scand. 6: 49—54.
(11) Lindberg, B. & Theander, O. 1952. Studies on *Sphagnum* peat. II. Lignin in *Sphagnum*. Ibid. 6: 311—312.
(12) Moon, F. E. & Abou-Raya, A. K. 1952. The lignin fraction of animal feeding stuffs. III. The determination of »total» lignin. J. Sci. Food Agric. 3: 595—608.
(13) Official methods of analysis of the association of official agricultural chemists. pp. 744—745. Wisconsin. 1950.
(14) Paloheimo, L. 1929. Beitrage zur Ligninbestimmung mit Säurehydrolyse. Biochem. Z. 214: 161—174.
(15) —»— & Paloheimo, I. 1949. On the estimation of the total of vegetable membrane substances. J. Scient. Agric. Soc. Finl. 21: 1—15.
(16) Phillips, M. & Davis, B. L. & Weihe, H. D. 1942. Composition of the tops and roots of the timothy plant at successive stages of growth. J. Agric. Res. 64: 533—546.
(17) —»— & Goss, M. J. 1935. Composition of the leaves and stalks of barley at successive stages of growth, with special reference to the formation of lignin. Ibid. 51: 301—319.
(18) —»— & Goss, M. J. 1939. Composition of the various parts of the oat plant at successive stages of growth, with special reference to the formation of lignin. Ibid. 59: 319—366.
(19) Russel, A. 1948. Interpretation of lignin. I. The synthesis of gymnosperm lignin. J. Am. Chem. Soc. 70: 1060—1064.
(20) Salo, M.-L. 1957. Lignin studies. I. Investigations concerning lignin determination. J. Scient. Agric. Soc. Finl. 29:

SELOSTUS:

LIGNIINITUTKIMUKSIA

II. LIGNIINIPITOISUUDESTA JA LIGNIININ OMINAISUUKSISTA ERILAISISSA KASVIAINEKSISSA

Maija-Liisa Salo

Yliopiston kotieläintieteen laitos, Helsinki

Tutkimuksessa on määritetty erilaisten kasviainesten ligniini-, kettoaine-, raakaproteiini- ja ligniinin metoksyylipitoisuus. Lisäksi on selvitelty eräitä ligniinin ominaisuuksia. Sekä kuiva-aineen että soluseinämäaineiden ligniinipitoisuus vaihtelee suuresti eri materiaaleissa, kuten taulukosta 1 käy ilmi. Ligniinin metoksyylipitoisuus on erilainen eri kasveissa, samassa kasvissa eri kasvuasteilla ja saman kasviyksilön eri solukoissa. Heinäkasvien ligniinistä liukenee vielä 0.1 N natriumhydroksidiliuokseen n. 50 %, mutta havupuiden ligniinistä 2 N liuokseenkin vain 2—6 %. Tavanomaisella happomenetelmällä eristetty ligniini sisältää todellisen hydrolyysijäännöksen lisäksi 72-prosenttiseen rikkihappoon liuennutta, mutta laimennettaessa saostunutta ainetta. Saostuneessa fraktiossa on typellisiä aineita suhteellisesti enemmän kuin ligniinipreparaatissa kokonaisuudessaan. Sen typettömäksi lasketun osan korkea metoksyylipitoisuus viittaa kuitenkin ligniiniin.
