A STUDY OF THE PROCESS OF LIGNIFICATION OF ONE-YEAR WILLOW WOOD

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This paper gives the results of a study of the processes of the formation of lignin in the wood of young willow shoots. The wood was studied in various stages of vegetative development in order to establish the best times for its collection.

In order to study the lignification of willow wood, we selected samples of willow of shrublike forms: Salix viminalis (basket willow) and Salix acutifolia (sharpleaf willow). These species are those found most frequently among domestic willow thickets.

Samples of the species mentioned were prepared from a willow plantation on sandy loam in the tree nursery of the Botanical Gardens of AS Belorussian SSR. No fertilizers were used in the growing of the willows. Samples of the willow wood were taken each year at definite times (Table 1).

Table 1

Data of collection of the wood	Salix vininalis			Salix acutifolia		
	yield of lignin by Popov's method	content of methoxyl groups		yield of lignin by Popov's method	content of methoxyl groups	
		in the wood	in the lignin		in the wood	in the lignin
1961						
27. V	25.47	1.62*	—	30.40	2.00*	—
26. VI	20.74	4.05	9.71	23.15	4.81	10.58
25. VII	21.83	5.03	11.81	25.25	5.37	10.43
25. IX	23.64	5.09	13.01	23.38	5.49	11.05
17. X	23.43	5.12	8.96	23.10	5.58	10.00
17. XI	23.05	5.85	11.60	22.25	5.52	12.17
20. XII	24.20	6.25	11.50	23.03	5.73	10.74
1962						
2. I	24.00	5.92	15.00	21.62	5.46	16.30
12. IV	24.93	5.21	5.60	21.22	5.06	5.73
27. VI	21.60	5.02	5.67	21.50	5.00	5.80
7. VII	22.75	5.34	5.96	22.44	5.01	5.86
18. VII	22.38	5.64	6.07	22.52	5.13	6.90
27. VII	23.77	5.67	6.79	23.72	5.19	7.08
12. X	21.53	5.91	9.60	23.95	6.08	8.03
1963						
12. I	23.31	6.02	15.98	24.09	5.96	15.61
27. VI	22.80	5.11	7.33	22.77	5.25	8.19
27. VII	25.21	5.86	9.80	23.77	5.61	8.94
2. XI	22.48	6.28	16.40	23.55	6.13	15.83

*In the wood and bark.

The lignin from the wood of the willow shoots was isolated by I. D. Popov's method with 37% hydrochloric acid [1]. Its yields proved to be high because of the partial humification of carbohydrates, forming lignin-like substances (pseudolignins) which were isolated together with the lignin [2]. The methoxyl groups in the wood and lignin of the willow were determined by Vielock and Schwappach's method [3]. These groups are the most characteristic among those present in lignin, they are found in lignin of all ages, and are attached directly to its aromatic nuclei [4].

The results of a quantitative determination of lignin and methoxyl groups (% on the weight of the absolutely dry material) are given in Table 1. The amounts of phenolic hydroxyl and carboxyl groups in the lignin isolated were determined by the chemosorption method [5]. It was found that in the lignin of the wood S. viminalis as it matured (material collected on July 25 and September 25, 1961), the amount of phenolic hydroxyl groups fell from 6.28 to 3.38 meq/g and that of carboxyl groups from 1.08 to 0.63 meq/g. In the lignin of S. acutifolia (from May 27 to September 25, 1961) the contents of these groups fell from 5.04 to 3.08 meq/g and from 1.11 to 0.78 meq/g, respectively. This shows that the lignin of the wood of more mature willow shoots is more highly polymerized [6].

The observation of the growth and development of the willow shoots and also the study of the chemical composition of the lignin showed that several physicochemical factors affect the process of the lignification of willow wood: moisture, illumination (shortened or lengthened day), and the time of vegetative development of the willow shoot. In the willow wood collected in 1962, in the same period of vegetative development, with a greater amount of atmospheric precipitation (718 mm) in the year, less lignin formed than in 1963 when the amount of atmospheric precipitation was 562 mm.

While on June 27, 1963, there was 22.8% of lignin and on July 27, 1963, 25.2% of lignin in the wood of S. viminalis, in 1962 at the same times of vegetative development the amounts of lignin were less, 21.6 and 23.8%. The same tendency was observed for willow of the species S. acutifolia.

To elucidate the influence of the length of illumination on the formation of lignin, several willow plants were kept in the dark from 8 a.m. to 5 p.m. The samples for investigation were taken simultaneously from plants which had been kept in the dark and plants which had not. Table 2 gives the results of these investigations (% by weight of absolutely dry material).

Table 2

Date of obtaining the wood	Day length	Amount of lignin by Popov's method	Methoxy groups	
			in the wood	in the lignin
July				
20	Normal	22.39	5.84	8.02
20	Shortened	22.63	5.95	8.18
30	Normal	22.13	5.67	10.24
30	Shortened	22.67	5.82	10.80
August				
10	Normal	23.42	5.33	11.58
10	Shortened	23.94	5.64	12.65
28	Normal	22.50	5.30	15.33
28	Shortened	23.90	6.05	16.13
September				
27	Normal	23.06	5.73	16.90
27	Shortened	23.16	6.20	18.15

It can be seen from Table 2 that with a decrease in the time of illumination the contents of lignin and OCH_3 groups in the willow wood increased considerably. It was established that in the wood and bark of willow shoots the content of readily-cleavable methoxyl groups increased somewhat in the shortened days [7].

We give the results of a determination of the easily-cleavable OCH_3 groups in samples collected on September 27, 1963:

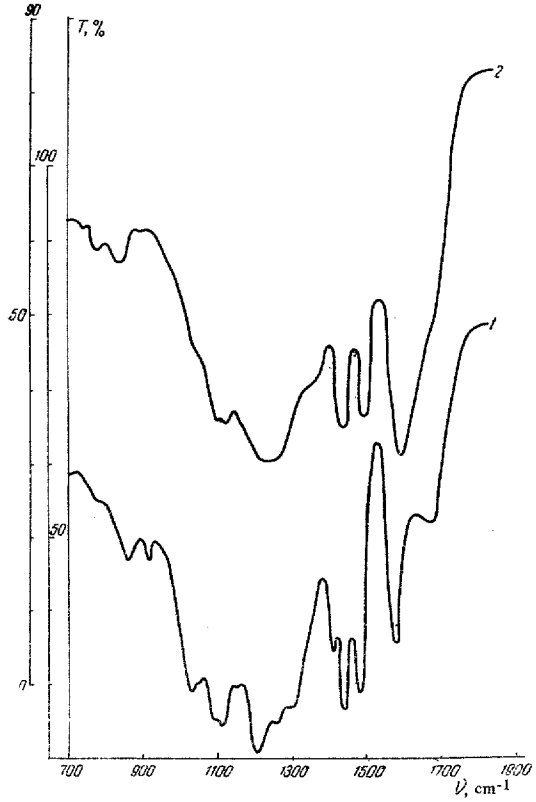
Sample	Day length	%
Wood	Normal	0.40
Wood	Shortened	0.42
Bark	Normal	0.41
Bark	Shortened	0.66

The chemical composition of the lignin as a function of the growth of the shoots was studied over three years. As can be seen from Table 1, the amounts of OCH_3 groups and lignin in the shoots depend on the process of their ageing. In the nonhydrolyzable residues of willow shoots methoxyl groups are present as early as April-May [8].

The presence of lignin in the wood of the shoots is confirmed by a study of the samples by IR spectroscopy (figure). For the lignin obtained from the May, June, and July shoots, the IR spectra are similar, all having a band at 1600 cm^{-1} due to aromatic rings. The IR spectrum of the lignin collected on January 12, 1961 differs from those described above: in place of two bands (1420 and 1460 cm^{-1}) a band has appeared at 1414 cm^{-1}, the band of the ether linkage (910 cm^{-1}) has disappeared, and a section of the 1700–1500 cm^{-1} bands has changed. Consequently, the lignin has changed qualitatively.

As the wood of the willow shoots forms (from May to January) the amount of OCH_3 groups in the willow wood and lignin increases (see Table 1). In both species of willow (from May to January) the content of lignin calculated from

the methoxyl numbers [9] rises.



IR spectra of lignin from the wood of S. viminalis collected on January 21, 1963 (1) and on January 12, 1963 (2).

To elucidate the participation of monosaccharides in the formation of willow lignin, the composition of the hydrolysates of the readily hydrolyzable polysaccharides of willow wood collected in January-April was considered. The hydrolysates were investigated for their content of monosaccharides by paper chromatography [10].

In July, at the moment of formation of lignin in the shoots, the amount of water-soluble carbohydrates decreases: the amount of reducing substances (RS) in aqueous extracts of S. acutifolia is 6.58% in June and only 1.32% in July. In the samples of willow studied, 75–90% of the RS consists of glucose, arabinose, and mannose. The amount of tanning substances in these samples is about 2% (on the weight of the absolutely dry wood). In June the amount of polysaccharides, glucose, fructose, and arabinose, in the willow hydrolysates falls considerably. The smallest amount of glucose is found in the hydrolysates of both species of willow at the end of May and the beginning and middle of June (S. viminalis, 2.31%, S. acutifolia, 1.33%), the smallest amount of fructose in the middle of July (0.24 and 0.11%), and the smallest amount of arabinose at the end of July (1.03 and 1.07%).

Likewise, the smallest amounts of glucose (1.33%) and of fructose (0.11%) were found in the hydrolysates of the wood of S. acutifolia collected on July 18, 1962, and the smallest amount of arabinose (1.07%) in that of July 25, 1962. Experiments with radioactive solutions of monoses showed that, in the synthesis of the lignin of the wood, glucose undergoes oxidation fastest, followed by xylose, mannose, and fructose [11].

It follows from the experimental results obtained that the process of lignification of willow wood takes place in the following way. With a change in the climatic conditions such as occurred in the dry year 1963, the synthesis of lignin and its precursors is intensified [12]. The limited supply of water led to a disturbance in the structure of the cell protoplasm, the colloids of which coagulated. Part of the polysaccharides formed underwent dehydration and other transformations and was left in the form of nonsugars [13]. While up to July the lignin had accumulated from precursors, by the end of the year, in October-January, when the days had become shorter, the metabolic processes had lessened and in the main the lignin had changed qualitatively. This is confirmed not only by the increase in the

content of methoxyl groups in it but also by the decrease in the amount of phenolic and carboxyl groups. Thus, the lignins of just-formed and mature willow woods are qualitatively different. The amount of aromatic substances in the lignin of wood collected in January is higher than that in the lignin of the shoots collected in July.

CONCLUSIONS

1. The content of lignin in the wood of Salix viminalis and Salix acutifolia increases with the growth of the shoots during shortened days and also in drier climatic periods.

2. The monosaccharides, glucose, fructose, and arabinose take part in the synthesis of the lignin of young wood of Salix viminalis and Salix acutifolia. The formation of lignin of one-year willow wood takes place from May to January (with a maximum in June-July). The content of methoxyl groups in it gradually rises.

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