CHANGES IN THE COMPOSITION OF THE NEEDLES OF PINUS AUSTRIACA LINK DURING THE AGEING-PROCESS

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In spring the young needles of coniferous trees begin to develop. They grow during summer and finish their growth in autumn. In one year they are practically full-grown, but remain at the tree during some years before they drop.

The growing zone is localized in the basal part of the needle. This zone is very small; already at a distance of about 1 cm from the base no growth occurs. Above this zone the needle is full-grown. This fact can be demonstrated from both the anatomical and chemical properties of the needle. Unfortunately it is not possible to measure the division of growth in the basal part because this part is wholly included by membranous scales. After removal of the scales it desiccates very soon. Neither it is possible to cover the base again with the scales after every measurement as desiccation and injury have seriously affected it.

Alexandrov and Djaparidze(1) demonstrated in microscopic sections that at a distance of about 1 cm from the base strong lignification of various cell walls takes place. The transition zone between the part of the needle with very young, growing tissue and the part with full-grown, partly lignified tissue is very short. In this zone very rapid anatomical and chemical alterations will take place, in other words rapid ageing-processes. The composition at the base will be quite different from that at the top. Substances building up the young cell walls will soon be replaced by or transformed into the final products of the walls of the full-grown cells. Figure 1 shows these changes in a very striking way.

This study intends an investigation of the differences in the chemical composition of various zones of the needles in order to arrive at valuable conclusions concerning the alterations which this composition undergoes during the ageing-processes.

Some data concerning the substances present in the full-grown needles are known. Kawalier(6) investigated the resins and water-soluble products. Cleve von Euler(2) studied the

resinous and tannic products which, according to her, should be closely related to lignin. Jaccard and Frey-Wyssling (5) determined the content of resins and of wax of needles (one and two years old), amounting resp. to 6—8 per cent and 1.3—2 per cent. Grosskopf (4) investigated the composition of the cell walls of full-grown, but still green needles and of decayed, browncoloured-ones in relation to humification problems. From this study it appears that green needles have a cellulose content of 26.3 per cent, a lignin content of 37.6 per cent and a pentosan content of 10.4 per cent, calculated on the dry matter. But it is extremely probable that Grosskopf determined tannins and humic products together with lignin.

Our investigation was performed with young needles of *Pinus austriaca* Link. They were gathered in June 1936 and their length varied from 4—7 cm. They were cut in four parts viz. *a*) the basal part 0.5—0.6 cm in length, *b*) the second zone of the same length, while the rest was cut again in two equal parts viz. *c*) the middle and *d*) the apical part each 1.5—3.0 cm in length.

The basal part, which is soft and colourless, is included by membranous scales (these scales, being strongly lignified, were not investigated). The second zone, partly covered by the scales, begins to harden and to colour. The rest of the needle is hard and green-coloured.

The samples of the four parts were dried at 40° C in vacuo and ground in a desintegrator. The percentage of the dry matter was determined and appeared to increase from base to top (table 1).

Before analyzing, the material was extracted at least with ether, but the ether-extractives were not investigated. In the ether-extracted material the following determinations were performed; extraction in a Soxhlett-apparatus with alcohol-benzene (1 : 1) during 20 hours; water-extraction during 3 hours; hydrolysis with dilute sulfuric acid (1 per cent) yielding red-coloured liquids especially from the younger parts; determinations of the ash content, of the reducing sugars in the water extracts; analyses of the cell wall substances viz. pectin, cellulose, hemicelluloses (pentosan) and lignin.

The percentage of pectic substances was determined by establishing the content of galacturonic acid. This was done by determination of the amount of carbon dioxide liberated by boiling the material with 12 per cent hydrochloric acid and multiplying this quantity with the factor 4.4 [method Lefèvre-Tollens-Sloep (9)].

Cellulose was determined according to the method of Kuerschner-Hoffer (7) by treatment of the material with an alcoholic

nitric acid solution (4 : 1) and hemicelluloses were found according to the destillation method of T o l l e n s (10), whereby furfural is split from pentosan and pectic matter by using 12 per cent hydrochloric acid.

The amount of lignin present in the material was found by treatment with 72 per cent sulfuric acid [method R i c h t e r (8)].

The determinations of the cell wall substances were performed in the samples, 1) after an ether-extraction, 2) moreover after application of an alcohol-benzene-extraction, 3) furthermore after a water-extraction and 4) after performing an acid-hydrolysis. We followed this procedure to study the influence of these treatments on the analytical results. It may be possible that during these treatments products are removed which otherwise are determined with the substance in question giving an impure image of the amount of this substance really present in the material. The analytical results are given in the tables 1—5 and in figure 1.

TABLE 1. Result of various extractions and of ash determinations performed with the needles of *Pinus austriaca.*

material	dry matter in fresh material	ether-extract calculated on dry original matter	% calculated on dry ether-extracted material			
			alc.-benzene extract	water extract	reducing sugars in water extract	ash
	%	%	%	%	%	%
basal part	21.70	8.30	30.50	41.70	2.50	4.65
second „	23.70	5.95	27.05	36.00	2.50	3.30
middle „	32.10	5.00	18.50	22.80	2.85	2.35
apical „	36.00	4.60	18.10	22.70	3.85	1.95

TABLE 2. Cellulose content in the needles of *Pinus austriaca.*

material	% calculated on dry ether-extracted material			
	after extraction with [1]:			
	ether	alc.-benzene	water	acid
	%	%	%	%
basal part	13.30	12.10	10.50	9.90
second „	22.00	20.50	20.05	16.30
middle „	30.50	30.20	27.60	26.40
apical „	31.45	30.50	28.05	26.35

[1] For these extractions see text-page 348.

TABLE 3. Content of pectin and pentosan in the needles of *Pinus austriaca.*

material	% calculated on dry ether-extracted material				
	pectin, calculated as tetra-galactu-ronic acid [3] (after ether extr.)	furfural phloroglucide [1] after extraction with: [2]			
		ether	alc.-benzene	water	acid
	%	%	%	%	%
basal part	5.70	5.75	—	3.50	0.45
second „	5.55	8.45	—	5.25	1.60
middle „	5.30	12.60	—	8.30	2.60
apical „	4.60	13.40	—	9.05	2.50

TABLE 4. Content of products insoluble in 72 % H_2SO_4 ("lignin") present in the needles of *Pinus austriaca.*

material	% calculated on dry ether-extracted material after extraction with: [2]			
	ether	alc.-benzene	water	acid
	%	%	%	%
basal part	34.60	32.30	28.50	19.80
second „	28.25	25.90	25.10	20.10
middle „	29.00	24.55	23.60	—
apical „	30.60	25.25	23.90	24.40

In the next table we give the amounts of material, disappeared after the successive extractions, calculated on the dry ether-extracted material.

TABLE 5. Amount of dissolved material after various extractions.

material	after alc.-benzene extraction	moreover after water-extraction	furthermore after acid-hydrolysis	final residue
	%	%	%	%
basal part	32.50	16.25	19.65	31.60
second „	28.80	11.90	19.30	40.00
middle „	19.50	8.70	22.30	49.50
apical „	19.00	7.70	16.95	56.35

[1] Furfural from pentosan together with pectin.
[2] For these extractions see text-page 348.
[3] 4 mol. galacturonic acid (4.4 × amount of CO_2 liberated) reduced by 3 mol. water.

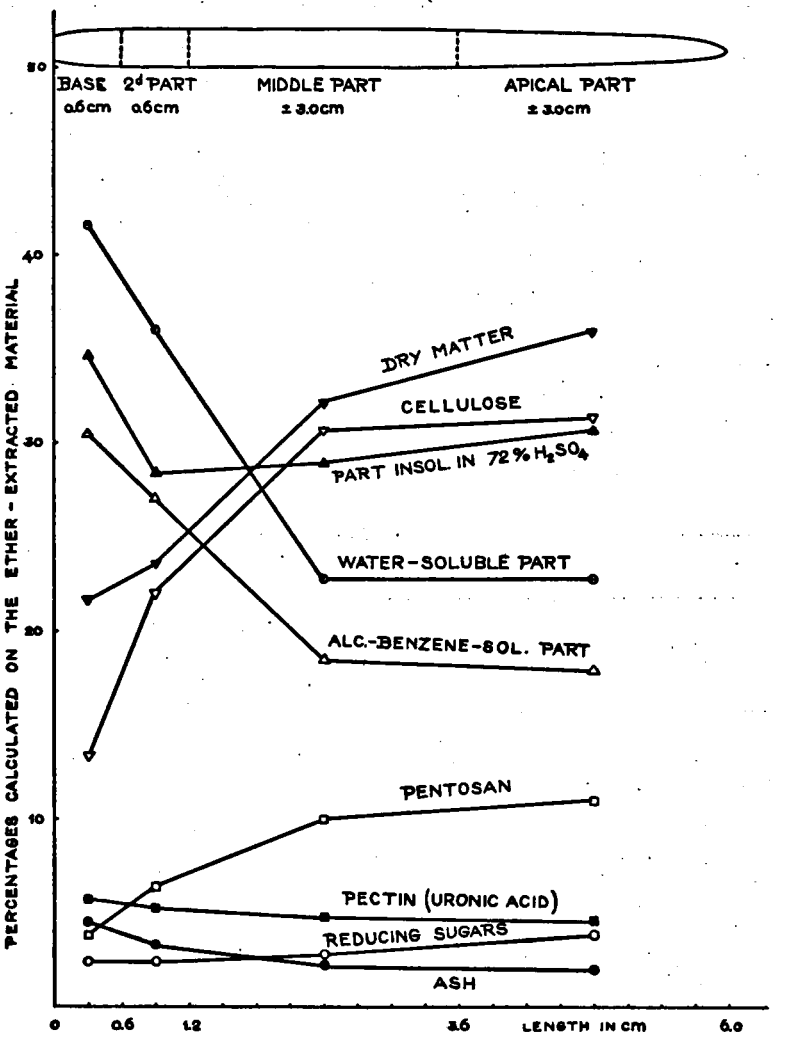

Fig. 1. Average chemical composition of the successive parts of the pine-needle.

The analytical results seem conclusive. In every respect important variations appear to exist in the chemical composition of the different parts of the needle. The data of middle and apical part diverge very little but they strongly vary from the second part and still more from the base.

It appears that the base consists of more soluble components and less insoluble cell wall substances than the other parts of the needle. The ether extract is much higher at the base than at the top. It is very probable that in the younger parts we deal with various ether-soluble, resinous and fatty products which polymerize afterwards and become insoluble. To a greater extent we can state this for the alcohol-benzene extracts. It is presumable that tannins play an important part in this case. Especially tannins from the catechol group are present in a large quantity. Unfortunately these compounds very strongly influence the determination of lignin, because they apparently give insoluble compounds with concentrated sulfuric acid.

The percentage of water-soluble products is high, especially in the basal part. However, various substances are soluble in water which are extracted also with alcohol-benzene. The lower percentage of reducing sugars in the water extract of the base is remarkable. These sugars may originate from the reserve-carbohydrates supplied from the stem to build up the cells and their walls in the young tissues. It is conceivable that the sugar content in the green-coloured parts of the needle may be higher as in these parts the CO_2-assimilation occurs.

As a result of the greater abundance of water and of the more intensive processes of development in the base, this part contains, therefore, a much higher percentage of inorganic salts.

It is very striking that the whole needle contains little pectin (calculated as a galacturonic acid complex). In this respect base and top vary very little. This is in contradistinction with the observations on other plant materials. Mostly the young parts contain a large amount of pectic substances which disappear for the greater part during ageing. For instance this fact is realized very well in the analysis of the sunflower stalk (3). In the needles of coniferous trees it is, therefore, not probable that pectin plays an important part in the ageing-processes.

In table 3 the data for furfural phloroglucide are given. This furfural originates from the pentosan and from the galacturonic acid of the pectin. It has been stated that 3 parts of galacturonic acid correspond with 1 part of furfural phloroglucide (9). We can subtract from the data from the third column of table 3 one third of the data from the second column of the same table. The results obtained,

converted with the aid of the tables of T o l l e n s, give the amounts of pentosan. At the base the content of this substance amounts to 3.9 per cent, at the second part to 6.35 per cent, at the middle part to 10.1 per cent and at the top to 11.0 per cent, calculated on the ether-extracted material. The data are much lower than those obtained with the sunflower stalk (3). As well as the coniferous wood the needles may contain also hexosans. The determination of these hemicelluloses, however, yielded too many difficulties and, moreover, the data appear to be unreliable as they include other substances together with hemicelluloses. A part of the furfural-yielding substances has disappeared from the material after water-treatment. The decrease of these products is still greater after acid-treatment. In this case the greater part of the hemicelluloses and pectins are hydrolysed and dissolved.

The cellulose content shows a conspicuous increase from base to top. After the alcohol-benzene-extraction the percentage of cellulose has remained practically constant (the basal part only shows a small decrease). Extraction with water and treatment with dilute acid also give a small diminution of this component. The decrease may be due to the removal of some hemicellulose by hydrolysis, for the determination of cellulose is not quite exact, including a part of the hemicelluloses.

To determine the lignin content the successive parts of the needles were treated with 72 per cent H_2SO_4 and the residue obtained was considered as "lignin". But the results are very peculiar and is it sure that we do not deal exclusively with lignin. At the first place this fact is shown by the amount of "lignin" found in the young base viz. 34.6 per cent, while this part has not been lignified at all! The alcohol-benzene-extraction does not diminish these data in a marked way (2—5 per cent, chiefly at the base); the water-extraction reduces them a little more, while finally the acid-hydrolysis produces a considerable decrease in the basal parts. After this last treatment the content of "lignin" at the base has been reduced from 34.6 to 19.8 per cent. By far the greater part, however, must be another substance, for the basal part can only contain a few per cents of lignin as is shown in microchemical way.

This microchemical study of the base demonstrated that only in the young vascular bundle a lignin reaction occurs with phloroglucinol and HCl. It appeared, however, that most cells contained a yellowish granular mass. This mass was stained intensive red after addition of an alcoholic vanillin solution and concentrated HCl. Ferric chloride only intensified the yellow colour of the mass. Moreover it appeared that 72 per cent H_2SO_4 did not dissolve the

mass. We deal here with a tannin of the catechol group and very probably with a complex of this tannin with other substances, perhaps with pectin. The tannin is present in a large quantity in the parenchyma cells, to a less extent in the young vascular bundle, in the cells surrounding the resin canals and in the hypodermal cells, while it is absent in the parenchyma within the endodermis. (N.B. In material preserved with alcohol the tannin permeates into the greater part of the cell walls.)

In older parts of the needle the content of this tannin decreased. The reaction with vanillin-HCl was less intensive than in the cells of the basal part. The percentage of lignin rapidly increased in the direction of the top; the cell walls of the epidermis, of the hypodermis, of the sclerenchyma surrounding the resin canals, of the vascular bundle and successively of the whole parenchyma within the endodermis lignified. These walls were stained intensive red with phloroglucinol-HCl (the outer wall of the epidermis, however, was stained yellow). Vanillin-HCl gave a yellow colour in many walls of epidermis, hypodermis and vascular bundle and a light red colour in the cell contents.

The residue from the basal parts obtained after treatment with 72 per cent H_2SO_4 contains, therefore, much insoluble matter combined with tannin and only a small amount of pure lignin while, on the contrary, the same residue from the apical part consists of much lignin and less tannic compounds. Unfortunately it was impossible to separate lignin and tannin quantitatively. Therefore, we could gather only qualitative data concerning the lignification of pine-needles. We do not know if the tannin we demonstrated in these needles is connected with the tannic compounds which C l e v e v o n E u l e r isolated from coniferous needles and which she considered as closely related to lignin.

If it were possible to accomplish a good separation between both components, we should be able to study the significance of the tannins present in the needles with regard to the lignification of these needles.

Summarizing we may state the following results:

The growing parts of the pine-needles are rich in water, in products soluble in ether and in alcohol-benzene, very rich in tannin, poor in cellulose, pentosan, lignin and pectin.

The full-grown parts which are present already at a short distance from the base have a rather constant composition during the ageing of the whole needle. The percentage of dry matter has been augmented and that of soluble compounds diminished. The content of cellulose and pentosan has been increased considerably, but the

percentage of pectin has not changed. Microchemical reactions showed an important increase of lignin and a considerable decrease of tannin.

In consequence of the lack of a good method to determine tannins apart from lignin in this material, it was not possible to give valuable information on the chemism of the lignification process.

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