THE STRUCTURE OF GALLOTANNINS

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Gallotannins can be used both in medical practice and in some branches of the national economy. Typical representatives of this group of substances are the tannins of Chinese galls (Rhus semialata) and Turkish galls (Gallae turcicea) and of the leaves of Sicilian sumac (Rhus coriora L.) and the common smoketree (Cotinus coggygria Scop.).

Fischer and Freudenberg [1, 2] showed that the tannin from Rhus semialata was based on a pentadigalloylglucose. Workers of many countries [3-9] have continued to develop the chemistry of natural tanning agents.

Schmidt [5] and Sissi et al. [10] have shown that the tannins from the galls of Rhus semialata and sumac leaves are closest in structure and that the tannin from Gallae turcicea appreciably differs from them.

As has been shown previously [10, 12], Fischer's Chinese tannin contained, in addition to the main substance, about 20% of gallic acid, 7% of m-digallic acid, and a small amount (traces) of two unidentified substances.

In 1966, a group of workers [11] using, in addition to chemical methods, NMR spectroscopy, established that the gallotannin from Rhus semialata consisted of 1,3,4,6-tetra-O-galloyl-β-D-glucose with an m-trigalloyl chain in position 2.

Recently, publications have appeared in which the results of a study of gallotannin and the substances accompanying it in the leaves of the sumac have been given [10, 12], and it has been shown that sumac tannin contains eight or nine galloyl residues.

The present paper discusses information relating to a determination of the structural features of the tannins of the leaves of sumac and the smoketree and of Gallae turcicea and Rhus semialata galls.

On acid or alkaline hydrolysis, the tannins form D-glucose and gallic acid (Table 1). Judging from the amount of gallic acid in the hydrolyzate (after the complete cleavage of the tannin) it can be shown that the tannins from smoketree and Chinese galls are the closest, each having an average of seven galloyl residues; in the tannin from sumac six acyl groups have been found, and in Turkish tannin five.

To determine the gallic acid residues in the materials investigated, in addition to chemical methods we used PMR spectroscopy (Fig. 1). For all four tannins the PMR spectra are similar in general outline and contain three groups of signals: 1) a broad signal with unresolved components having its center at 4.38-4.28 ppm due to the three protons of a sugar residue; 2) signals of a multiplet broadened group with poorly resolved components with its center at 5.8 ppm, assigned to the other four protons of the sugar component; and 3) a complex, well-resolved, multiplet with its center at 7.19 ppm due to the aromatic protons of galloyl residues. For the sumac, smoketree, and Chinese gall tannins, the signals of the sugar protons are identical and the ratio of the intensities of the first group of signals to those of the second is close to $\frac{3}{4}$. We assume that the first group of signals is due to the protons of a methylene group and an equatorial proton in position 5, and the second group to axial protons in positions 1, 2, 3, and 4. In Turkish tannin the ratio of the two groups of signals is somewhat different – $\frac{4}{3}$ – which may apparently be due to the presence in it of hydroxy groups not substituted by galloyl residues.

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TABLE 1. Results of a Determination of the Number of Galloyl Residues in the Gallotannins

Source of the gallotannin	Region of signals for definite multiplet groups of aromatic protons, ppm	No. of galloyl residues	Total no. of galloyl residues	Amt. of gallic acid in a hydrolyzate (determined by the chromatospectrophotometric method [13])	
				%	No. of galloyl res.
Sumac	7.75-7.43*	0.5	6.16	92-93	6.21
	7.43-7.11†	3.81			
	7.11-6.85‡	1.85			
Smoketree	7.65-7.40*	0.8	7.35	95-96	7.15
	7.40-7.10†	4.2			
	7.10-6.95‡	2.3			
Turkish galls	7.80-7.37*	1.04	4.60	87-89	4.91
	7.37-7.12†	2.06			
	7.12-6.90‡	1.40			
Chinese galls	7.75-7.42*	1.25	7.40	95-97	7.28
	7.42-7.10†	4.45			
	7.10-6.90‡	1.70			

* Intermediate galloyl residues.

† Galloyl residues attached directly to the carbohydrate components.

‡ Terminal galloyl residues.

To determine the number of galloyl residues from the integral spectrum we used the signals of the sugar protons as an internal standard (see Table 1). The figures of the table show that the number of galloyl residues in the various tannins varies from 4.6 to 7.4. The results of spectral determinations agree well with those of the chemical method and with literature information for Chinese tannin [11]. Britton et al. [11] were the first to attempt to assign the signals in the aromatic part of the spectrum of the methoxy derivative of Chinese gallotannin to the protons of the galloyl residues occupying nonequivalent positions in the molecule, i.e., terminal, intermediate, and attached directly to the sugar component.

In the PMR spectra of the trimethylsilyl derivatives of the tannins, which we have studied, there are likewise three clear multiplet groups of signals in the region of aromatic protons (see Fig. 1 and Table 1). It is natural to assume that the protons of the intermediate galloyl residues, i.e., those located in the center of a polygalloyl chain, undergo the greatest screening and therefore their signals should be present in the weakest field. Thus, the protons of the intermediate galloyl residues of different samples of tannins give signals at 7.80-7.37 ppm. The signals of the protons of the terminal galloyl residues are found in the 7.12-6.85-ppm region.

As compared with the results of Britton et al. [11], the protons of the terminal galloyl residues give signals in a stronger field, which is apparently due to the influence of the trimethylsilyl groups.

From the integral spectrum, a calculation was made of the number of nonequivalent units of galloyl residues. It can be seen from Table 1 that the number of residues directly attached to the glucose varies from 2.06 to 4.45, the number of intermediate residues from 0.5 to 1.25, and the number of terminal residues from 1.40 to 2.3.

The most important step in the study of the chemistry of the tannins is the determination of the degree of esterification of the carbohydrate component with galloyl residues.

After the exhaustive methylation of all four samples of tannin and subsequent alkaline hydrolysis of them, 3,4-dimethoxygallic acid (mp 167°C, λ_{max} 262 nm, mol. wt. 198.5) and 3,4,5-trimethoxygallic acid (mp 197-198°C, λ_{max} 267 nm, mol. wt. 213.5) were isolated. The carbohydrate residues of the methylated tannins from smoketree and Turkish galls were reduced with Fehling's solution, which shows that the semiacetal hydroxyls are substituted by galloyl residues, while the carbohydrate moiety of the tannins from sumac and Chinese galls did not possess reducing properties. This confirms that the semiacetal hydroxyls are not substituted by galloyl residues.

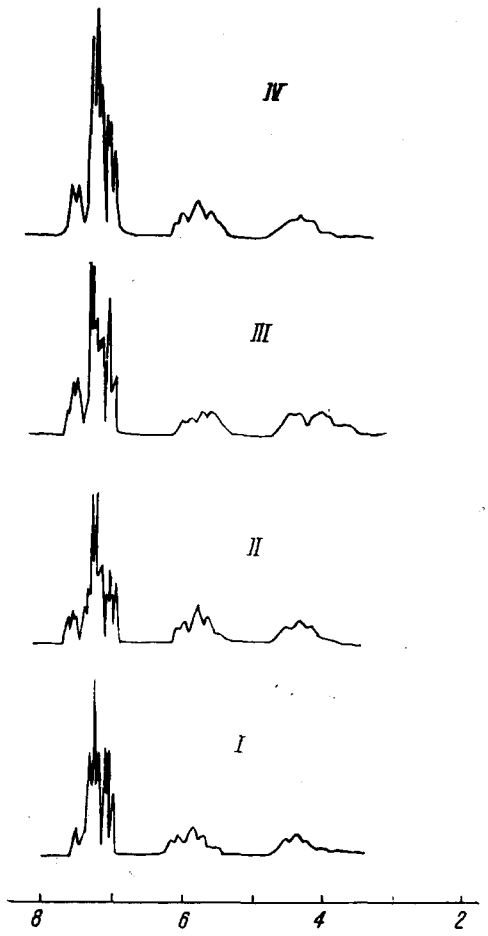

Fig. 1. PMR spectra of the gallotannins of sumac (I), smoketree (II), Turkish galls (III), and Chinese galls (IV).

It was found by acid hydrolysis of the carbohydrate residues of the methylated tannins that the sugar from sumac and Chinese galls gives a positive Fehling reaction and shows the presence of free D-glucose on chromatograms. Consequently, all the hydroxy groups of the carbohydrate components of these tannins are substituted by galloyl residues, with the exception of the semiacetal hydroxyl. The sugar residues of the tannins from the smoketree and Turkish galls differ from D-glucose, which shows the methylation of one of the alcoholic hydroxy groups.

The results of a comparison with an authentic sample of methylated glucose showed that for the tannins from the smoketree and Turkish galls the carbohydrate component is 3-O-methyl-D-glucose in each case.

EXPERIMENTAL

Preparation of the Samples of the Tannins. The gallotannin was extracted from the comminuted leaves of sumac and the smoketree with water and was deposited on a column of polyamide sorbent. The eluates containing the tannin were treated with ethyl acetate, and the ester extract was evaporated to small volume and precipitated with methylene chloride.

The tannin was likewise extracted from the Turkish and Chinese galls with water; they were transferred into ethyl acetate and purified by the method described by Makarevich et al. [14].

The individuality of the tannins was checked by two-dimensional paper chromatography. The samples were dried over phosphorus pentoxide in vacuum (10^{-2} mm Hg) at 110°C.

Quantitative Acid Hydrolysis of the Tannins. An accurately weighed sample of 1.000 g of the tannin was hydrolyzed with 20 ml of 5% sulfuric acid in the boiling-water bath for 10 h. The completeness of hydrolysis was checked by paper chromatography. The amount of gallic acid in the hydrolyzate was determined by the chromatospectrophotometric method [13]. To obtain the carbohydrate components, the hydrolyzates were treated with AV-17 anion-exchange resin (in the OH^- form) until they were neutral, the gallic acid was extracted with diethyl ether, and the aqueous residue was evaporated to 0.5-1.0 ml and chromatographed with an authentic sample of D-glucose.

Methylation of the Tannins. A sample of tannin (3 g) was dissolved in 150 ml of absolute acetone, and 7 g of potassium carbonate and 2 ml of dimethyl sulfate were added. The mixture was stirred at 40-45°C for 14 h and filtered, the solvent was distilled off, the oily residue was dissolved in 20 ml of methyl iodide, 0.2 g of silver oxide was added, and the mixture was again heated (40-45°C) with continuous stirring for 14 h. The reaction mixture was then filtered and evaporated to an oily residue, which was dissolved in chloroform and was purified on a column of alumina. The eluates were evaporated, the dry residue was dissolved in 5 ml of acetone, 25 ml of 5% caustic soda solution was added, and hydrolysis was performed on the water bath for 7 h. The hydrolyzates were treated first with a cation-exchange resin (in the H^+ form) and then with an anion-exchange resin (in the OH^- form). The methoxy derivatives of gallic acid were extracted from the neutral solutions with diethyl ether, and the aqueous residue was concentrated to 1-2 ml and subjected to Fehling's reaction.

Recording of the PMR Spectra of the Tannins. For this purpose, their trimethylsilyl ethers were prepared by the method described by Mabry et al. [15]. The spectra were recorded on a Hitachi-Perkin-Elmer R-20A instrument with a working frequency of 60 MHz. Carbon tetrachloride was used as the solvent and tetramethylsilane as internal standard.

SUMMARY

1. It has been established that the semiacetal hydroxyls of the carbohydrate components of the tannins of the smoketree and of Turkish galls are substituted by galloyl residues and the C_3 hydroxy group is free; on the other hand, in the tannins from sumac and Chinese galls all the hydroxy groups of the sugars are substituted by galloyl residues with the exception of the semiacetal hydroxyl.

2. It has been found that in the tannin from sumac, of the six gallic acid residues four are in the form of digalloyl and two in the form of monogalloyl groups; in the tannins from the smoketree and Chinese galls, of the seven gallic acid residues three are in the form of a trigalloyl, two of a digalloyl, and two of monogalloyl groups. In the tannin from Turkish galls, of the five gallic acid residues three are in the form of a trigalloyl and two of a digalloyl residue.

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