Polymer Structure of Commercial Hydrolyzable Tannins by Matrix-Assisted Laser Desorption/ Ionization-Time-of-Flight Mass Spectrometry

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ABSTRACT: The structures of six commercial hydrolyzable tannins, chestnut, oak, tara, sumach, chinese gall, and turkey gall tannins have been examined by matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry. Their oligomeric structures and structure distributions have been defined. Degradation products of rather different structure than what previously reported were present. Different galloyl glucose monomers were observed for chestnut and oak tannin extracts and in chinese gall gallotannin extract. Combination of positive- and negative-mode MALDI-TOF showed that most galloyl residues of the galloyl glucose chains were stripped from a skeletal glucose chain. Oligomers, in some cases up to 16 or 17 glucose units long, almost totally stripped of galloyl residues were observed. This indicated that a wide distribution up to very long galloylglucose chains exist in most commercial hydrolyzable tannin extracts. This indicated that these commercial tannin extracts are mainly composed of long galloyl glucose chains of mixed di-, tri-, and pentagalloyl glucose repeating units being present in the same chain. The presence of long glucose chains where most of the galloyl residues have been stripped indicates that their linkage may be sugar residue to sugar residue. Commercial tara and turkey gall tannins have been shown to be mainly polygallic oligomers of up to eight gallic acid residues linked to each other in a chain. Commercial sumach extract revealed itself a more complex mixture of glucose oligomers up to 13 repeating units. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 3847–3859, 2009

Key words: structure; hydrolyzable tannins; MALDI; oligomers distribution; chestnut; oak; tara; chinese gall; turkey gall; sumach

INTRODUCTION

Vegetable tannins have been used to tan leather either alone or accompanying other tanning agents for several thousand years. They are natural products obtained from plants and are very diffuse in the whole plant kingdom. The term natural vegetable tannins is used loosely to define two broad classes of chemical compounds of mainly phenolic nature, namely, condensed or polyflavonoid tannins and hydrolyzable tannins. To the recognized oligomeric nature of condensed tannins[1–4] corresponds the mixed nature of hydrolyzable tannins in which predominantly nonpolymeric but also polymeric structures are present.[2–5] Hydrolyzable tannins, including chestnut, tara, sumach, chinese gall, turkey gall, oak, and some other commercial tannin extracts are of two types: (1) mixtures of sugars with oligomers of simple phenols such as gallic and digallic acids, and (2) ellagitannins mainly formed of esters of a sugar, mainly glucose, with gallic and digallic acids, and with more complex structures containing ellagic acid. Many studies on the structure of these tannins have been carried out and in general their structure is known.[2–9] However, the structure of commercial, industrially extracted tannins appears to differ from what shown in the literature, the process of industrial extraction quite clearly changing the composition of the extract. Except for a very few articles on the structure of one major commercial hydrolyzable tannin extract, namely, chestnut wood tannin extract,[2,5] the structure of the other commercial tannins has not been reported, especially the type of oligomers present and their distribution. Thus, studies on chestnut tannin extract,[1,2] an ellagitannin, identified that its main constituents are castalagin (**I**) and vescalagin, positional isomers of identical 935 mass, composing, respectively, 14.2% and 16.2% by

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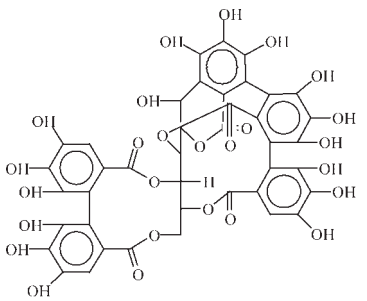
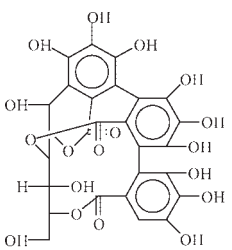
mass of chestnut tannin and the structure of which is as follows.



I

The rest of the tannin is composed of 6.6% castalin and vescalin (positional isomers of structure (**II**)),[1,2] 6% gallic acid, and 3% pentagalloyl glucose monomer.



II

However, between 25 and 50%,[1,5] is formed by a fraction that has been found to be a series of much higher molecular mass pentagalloyl glucose oligomers. The trimer of this was already identified.[5] Previous work sustained that structures of type (**I**) and (**II**) were only degradation products of the original structure of the tannin.[5]

As different gallic and ellagic tannins exist and are sold commercially, this article concentrates on the structure of four of the most common commercial gallic tannins, namely, (1) tara, chinese gall, turkey gall, and sumach, and of two mixed commercial galloellagic tannins, namely, (2) chestnut and oak bark tannins.

Since its introduction by Karas and coworkers in 1987,[10] matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) has greatly expanded the use of mass spectrometry toward large molecules and has revealed itself to be a powerful method for the characterization of both synthetic and natural polymers.[11–17]

Fragmentation of analyte molecules on laser irradiation can be substantially reduced by embedding them in a light absorbing matrix. As a result, intact analyte molecules are desorbed and ionized along with the matrix and can be analyzed in a mass spectrometer. This soft ionization technique is mostly combined with time-of-flight (TOF) mass analyzers. This is so because TOF-MS presents the advantage of being capable of providing a complete mass spectrum per event, for its virtually unlimited mass range, for the small amount of analyte necessary and the relatively low cost of the equipment.

MATERIALS AND METHODS

Tannin types

Six types of commercial hydrolyzable tannin extracts were used for MALDI-TOF analysis, namely, (1) Sumach (*Rhus coraria*) leaves commercial natural tannin extract, a gallotannin; (2) tara (*Caesalpina spinosa*) tannin, solvent-extracted with ethylacetate/acetone/water to eliminate the sugar fraction, a commercial natural tannin extract, a gallotannin; (3) chinese gall, the abnormal growth (gall) produced by parasitic aphids of *Melaphis chinensis*; a commercial natural tannin extract, a gallotannin, it is obtained from the galls solvent-extracted with ethylacetate/acetone/water to eliminate the sugar fraction; (4) turkey gall, the growth (gall) on the stems and branches of certain oaks (*Quercus spp*.) produced by a wasp (*Cynips tinctoria*); a commercial natural tannin extract, a gallotannin, it is solvent-extracted with ethylacetate/acetone/water from the galls to eliminate the sugar fraction; (5) commercial chestnut (*Castanea sativa*) wood tannin, water-extracted, and cold-sulfited with 3.5% ammonium sulfite, a galloellagitannin; (6) commercial Oak (*Quercus spp*.) extract both water and solvent extracted. All these tannins were commercial tannins supplied by Silva Chimica (S.Michele Mondovi', Italy).

MALDI-TOF-MS

The spectra were recorded on a KRATOS Kompact MALDI AXIMA TOF 2 instrument (Kratos Analytical, Shimadzu Europe Ltd., Manchester, UK). The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The length of one laser pulse was 3 ns. The measurements were carried out by using the following conditions: polarity-positive, flight path-linear, mass-high (20 kV acceleration voltage), 100–150 pulses per spectrum. The delayed extraction technique was used applying delay times of 200–800 ns.

MALDI-TOF sample preparation

Positive mode

The polymer samples were dissolved in acetone (4 mg/mL, 50/50% volume). The sample solutions were mixed with an acetone solution (10 mg/mL in acetone) of the matrix. As matrix, 2,5-dihydroxy benzoic acid was used. For the enhancement of ion formation, NaCl was added to the matrix (10 mg/mL in water). The solutions of the sample and the matrix were mixed in the proportions of 3 parts matrix

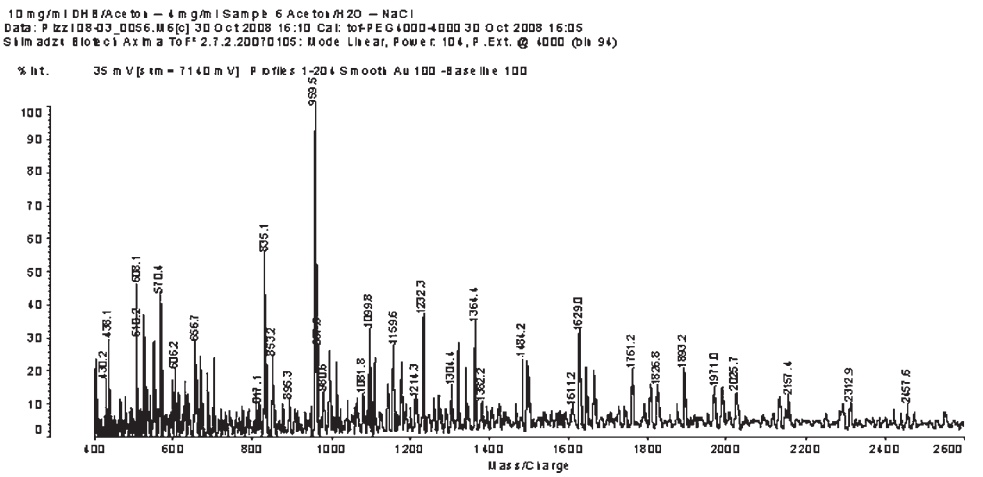


Figure 1 MALDI-TOF positive ion mode spectrum of water extracted chestnut tannin extract in the 400–2600 Da mass range.

solution + 3 parts polymer solution + 1 part NaCl solution and 0.5 to 1 μL of the resulting solution mix were placed on the MALDI target. After evaporation of the solvent, the MALDI target was introduced into the spectrometer by dry droplet sample preparation method. To each peak value in the resulting positive mode spectrum must be subtracted 23 Da of the Na^+ of the matrix to obtain the molecular weight of the chemical species of the peak.

Negative mode

The polymer samples were dissolved in acetone/water (4 mg/mL, 50/50% volume). The sample solutions were mixed with an tetrahydrofuran solution (10 mg/mL in acetone) of the matrix. As the matrix, Harmin was used. The solutions of the sample and the matrix were mixed in equal proportions and the resulting solution mix was placed on the MALDI target. After evaporation of the solvent, the MALDI target was introduced into the spectrometer by dry droplet sample preparation method. The peak value in the resulting negative mode spectrum is the molecular weight of the chemical species of the peak, as the matrix used does not interfere.

RESULTS AND DISCUSSION

Different types of hydrolyzable tannins, namely, ellagitannins and gallotannins, exist. Gallotannins in their polygallic form are often simpler oligomeric structures derived by the polymerization of gallic and digallic acids. To the gallotannins belong sumach, tara, chinese gall, and turkey gall tannins. Chestnut and oak tannnins instead are mixed gallic and ellagitannins.

A rather different structure and structure distribution became evident for commercial hydrolyzable tannins as shown by the chestnut tannin and oak tannin MALDI-TOF spectra in Figures 1–3. The

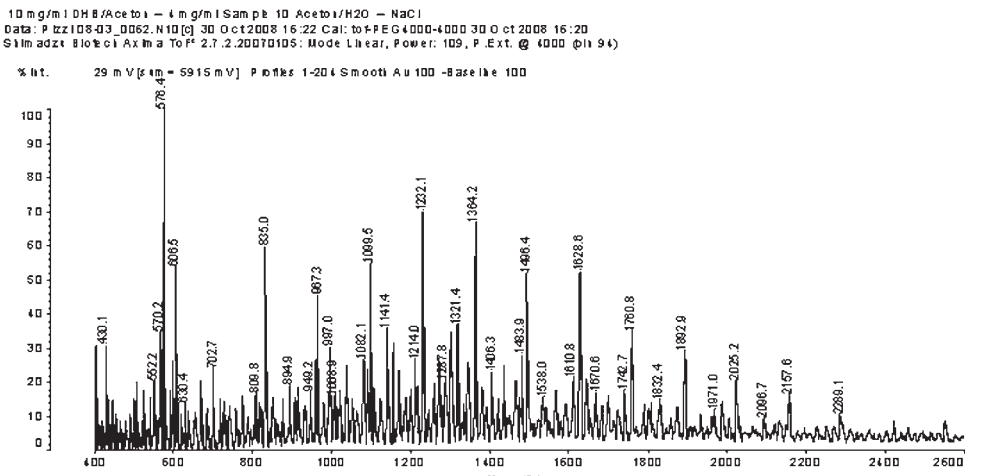


Figure 2 MALDI-TOF positive ion mode spectrum of water extracted oak tannin extract in the 400–2600 Da mass range.

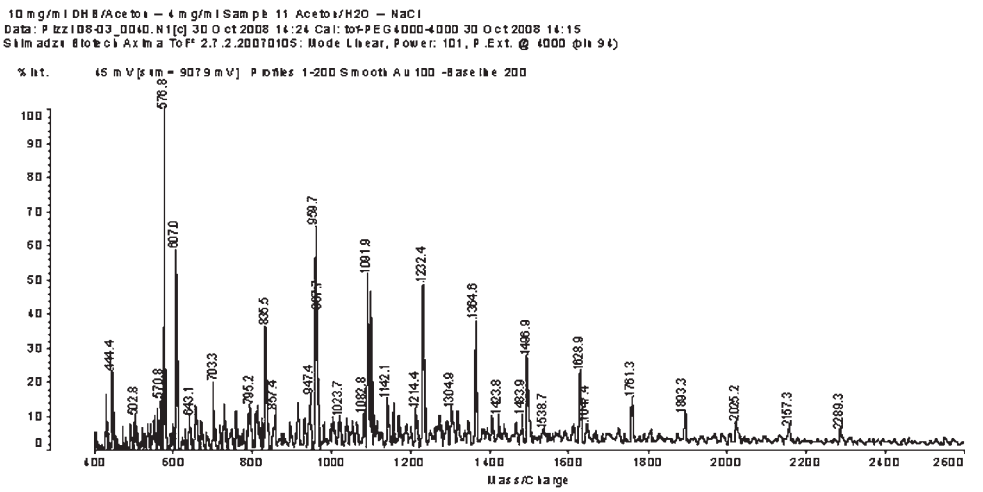


Figure 3 MALDI-TOF positive ion mode spectrum of solvent extracted oak tannin extract in the 400–2600 Da mass range.

structure of natural water-extracted chestnut tannin obtained by MALDI-TOF has already been reported.[5] It was found to be composed of rearranged molecules such castalagine and vescaline and predominantly of oligomers, up to trimers, of pentagalloyl glucose.[5] With the exception of the previously unknown pentagalloyl glucose oligomers presence all that was found by MALDI-TOF corresponds to what was obtained by other methods of analysis.[2] However, a second commercial product is extensively used, namely, water-extracted chestnut tannin treated with 3.5% ammonium sulfite after extraction to improve solubility and hide penetration in leather making. It is this chestnut extract type the MALDI analysis of which is reported in this article. This material should show the same type of spectrum as reported previously.[5] However, its MALDI spectrum in Figure 1 is rather different from that of natural chestnut tannin reported previously and presents a repeating motive of 132 Da. There is no possible gallic acid, digallic acid, galloyl, or ellagic acid residue that can correspond to such a repeating unit. It was finally realized that 132 Da is a monosaccharide glucose at 180 Da from which three hydroxy groups have been eliminated ($16 \times 3 = 48$; $180 - 48 = 132$ Da). Only 16 Da need to be subtracted because as the —OH is lost an —H is introduced on the same site of the molecule. This is then a glucopyranose ring residue from which any galloyl esters have been stripped. The alternative explanation that the 132-Da unit is formed by the loss of a HCHO molecule (30 Da) plus one molecule of water (18 Da) is possible but unlikely starting from the close pyranose ring configuration of glucose present in pentagalloyl glucose. Thus, the series of peaks present in Figure 1 corresponds to a series of glucoses oligomers up to 16 glucose residues long. To each of these oligomers is linked a single galloyl residue, one per oligomer chain only. The only oligomers confirming that this was the original configuration of this tannin are the peaks at 508 Da (digalloylglucose) and at 656 Da (trigalloys glucose) who are small but present in the spectrum. The sequence of peaks corresponding to these long carbohydrate chains observed in Figure 1 starts with the 438-Da peak (1 galloyl residue + 2 glucose residues stripped of —OHs, the 132 Da repeating unit). Every peak that follows adds a further galloyl-residues-stripped glucose repeating unit. Thus, starting from the 438-Da peak one can observe a series of peaks as listed in Table I.

These are not hemicelluloses or cellulose fragments; if this was the case, the repeating unit would be of 178–180 Da, and this is not the case.

This result is unexpected and merits discussion in depth. In MALDI-TOF, one can obtain positive ions and negative ions spectra. The one shown in Figure 1 is the positive ions spectrum. The negative ions spectrum is shown in Figure 4. The only two peaks of any note that are observed in Figure 4 are those of ellagic acid at 302 Da (negative ion peaks are not subtracted of the 23 Da for Na^+, as a different matrix is used) or of a digallic acid residue, and the peak at 483.5 Da, an ellagic or digallic acid residue linked to a glucose. There are traces of a couple of other compounds. These are at 631.5 Da, a monomer composed of a glucose to which are linked an ellagic acid residue and a gallic acid residue, and traces of pentagalloyl glucose at 933 Da. The 1086-Da peak indicates the presence of traces of a pentagalloyl glucose, to one of the galloyl residues of which is linked a sixth galloyl residue stripped from another pentagalloyl glucose. In fact, only the 300- to 302-Da peaks corresponding to ellagic or digallic acid residues appear in the negative mode spectrum after the galloellagic esters have been detached from pentagalloyl glucose.

TABLE I
Percentage Distribution of the Different Oligomers Present in Commercial Chestnut Extract (from MALDI-TOF Positive Ion Mode Spectra)

Da	Peak intensity	Relative abundance (%)	
438	32	5.3	1 Galloyl residue + 2 stripped glucose residues = dimer
508	48	7.9	Digalloyl glucose, monomer
570	44	7.3	1 Galloyl residue + 3 stripped glucose residues = trimer
657	30	5.0	Trigalloyl glucose, monomer
835	58	9.6	1 Galloyl residue + 5 stripped glucose residues = pentamer
959	100	16.5	Pentagalloyl glucose, monomer
967	30	5.0	1 Galloyl residue + 6 stripped glucose residues = hexamer
1099	34	5.6	1 Galloyl residue + 7 stripped glucose residues = heptamer
1232	38	6.3	1 Galloyl residue + 8 stripped glucose residues = octamer
1364	34	5.6	1 Galloyl residue + 9 stripped glucose residues = nonamer
1496	23	3.8	1 Galloyl residue + 10 stripped glucose residues = decamer
1629	35	5.8	1 Galloyl residue + 11 stripped glucose residues = undecamer
1761	22	3.6	1 Galloyl residue + 12 stripped glucose residues = dodecamer
1893	22	3.6	1 Galloyl residue + 13 stripped glucose residues = tridecamer
2025	14	2.3	1 Galloyl residue + 14 stripped glucose residues = tetradecamer
2157	14	2.3	1 Galloyl residue + 15 stripped glucose residues = pentadecamer
2289	13	2.2	1 Galloyl residue + 16 stripped glucose residues = hexadecamer
2312	14	2.3	2 Galloyl residues + 15 stripped glucose residues = pentadecamer

In previous work natural chestnut tannin extraction appeared to cause tannin rearrangement to products such as castalagine/vescalagine and castaline/vescaline.[5] The presence of pentagalloylglucose trimers constituted up to 60% of the extract. This indicated that extraction may cause the cleavage of longer pentagalloylglucose chains. This implied the rather novel concept that such a tannin is present, *in situ* in the wood, as a pervasive and extended random tridimensional macromolecular network of pentagalloylglucose chains, capable of being extracted exclusively by its degradation.[5] The MALDI-TOF spectra in Figures 1 and 4 appear to support such a hypothesis. The cleavage and elimination of the galloyl ester residues from the spectrum then shows the full length of the skeletal glucose chain on which these were originally linked. This occurs because the glucose chains, once stripped of their galloyl residues, are within the range of masses observable by the MALDI-TOF technique. It indicates that pentagalloylglucose oligomers of up to 16 repeating units are present in this commercial tannin extract. This supports the hypothesis that such a tannin may be present in the wood as an extended macromolecular chain.[5] The only oligomers in Figure 1 confirming that this is the correct interpretation, and the original configuration of this tannin, are the peaks at 508 Da (digalloylglucose = 23 (Na^+) + 152 × 2 + 180 = 507 Da), at 656 Da (trigalloylglucose, 657 Da) who are small but nonetheless present, and the dominant 960-Da peak of pentagalloyl glucose itself (23 (Na^+) + 152 × 5 + (180 − 3) = 960 Da). The series of

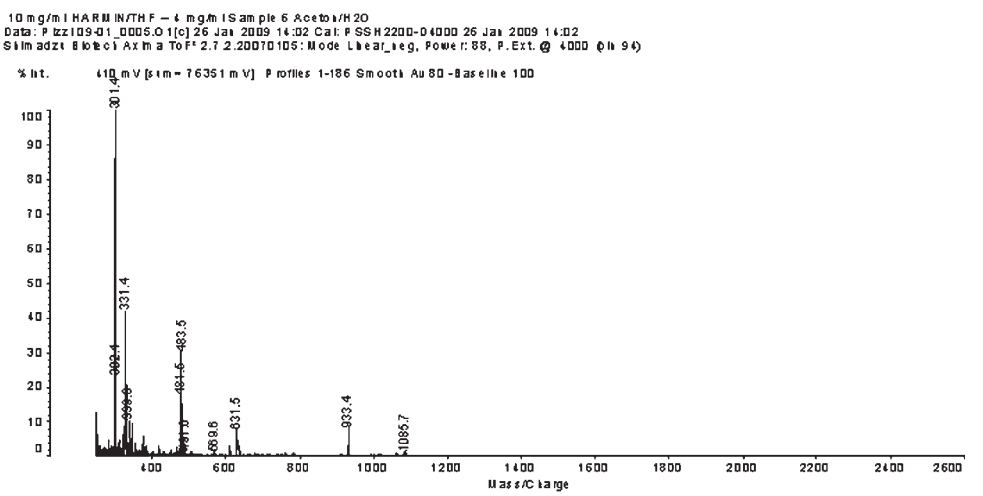


Figure 4 MALDI-TOF negative ion mode spectrum of water extracted chestnut tannin extract in the 400–2600 Da mass range.

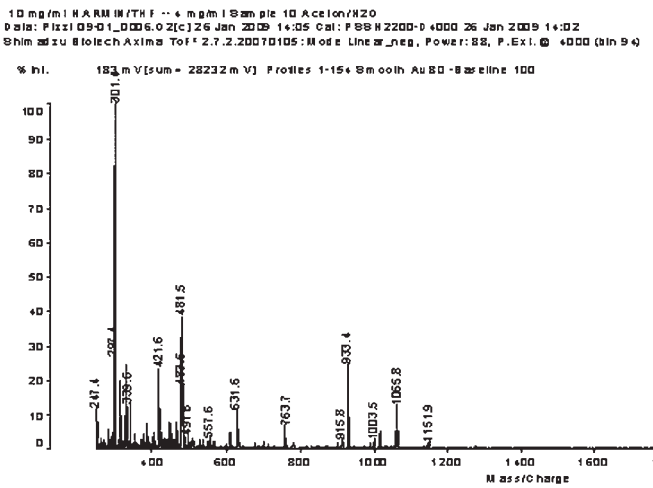
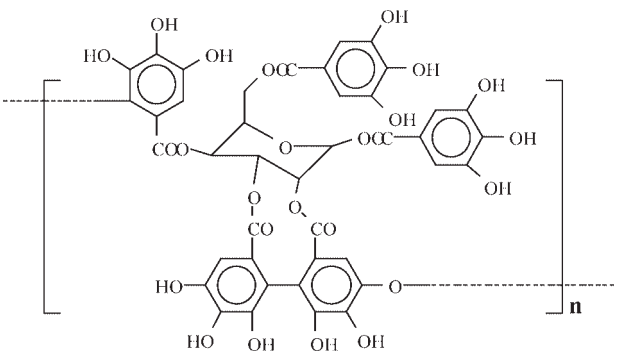


Figure 5 MALDI-TOF negative ion mode spectrum of water-extracted oak tannin extract in the 400–2600 Da mass range.

oligomers present in this tannin derived from the MALDI-TOF spectrum in Figure 1 are shown in Table I.

The same characteristics are noted in Figures 2 and 3 showing the MALDI-TOF spectrum for water-extracted and solvent extracted oak tannin. The pattern in Figure 3 of the solvent-extracted oak tannin is the clearer one. The predominant repeating unit is again 132 Da; hence, glucose stripped of its –OH groups and of its five galloellagic residues. The single pentagalloylglucose peak at 960 Da is again present and is the second most intense one. To this is associated the 1091-Da peak formed by one stripped glucose residue linked to the pentagalloyl glucose. In this spectrum (Fig. 3), however, the dominant peaks are the 577-Da and its associated 607-Da peak. These are, respectively, a glucose trimer in which the —OH at the C6 has been stripped but the $—CH_2^+$ still remains attached (577 Da) and the same glucose trimer where another complete C6 group ($—CH_2OH$) is still attached. The series of peaks is then 703 Da (three glucoses linked to a single galloyl residue), and a series of oligomers at 836, 968, 1100, 1232 (dominant of this series), 1364, 1496, 1628, 1761, 1893, 2025, 2157, and 2289 Da. Thus, one galloyl residue linked to a chain of 15 glucoses. In the water-extracted oak tannin in Figure 2 even a small 2421-Da peak is present. This corresponds to a 16-glucoses oligomer. The negative ion mode MALDI-TOF spectrum of the water-extracted oak tannin is shown in Figure 5. The trend is the same as in Figure 4: only monomeric species stripped from the main sugar chain shown in the positive ion mode in Figure 2 are present. The series of oligomers present in this tannin derived from the MALDI-TOF spectrum in Figure 3 are shown in Table II.

Thus, the structures that can be proposed for the main constituents of these two galloellagic hydrolyzable tannins is that of mixtures of oligomers up to 15 to 16 repeating units. The original repeating unit of these is pentagalloyl glucose (**I**). However, industrial extraction has also subtracted or hydrolyzed some galloyl residues from the main chain. Its appearance then is that of a mix of digalloyl, trigalloyl, and pentagalloyl glucoses linked together in oligomers 15 to 16 repeating units long, structure (**III**), where n varies between 1 and 16. These galloyl residues are still present in the extract, they are part of it.



III

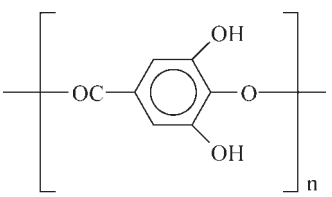
The implication of this is that the repeating units in galloyl glucose chains may be linked differently to each other and then according to the accepted model of structure (**III**).[5,18,19] This is clear as in the positive mode spectrum the long glucose chains are linked to each other also when galloyl residues have been stripped away. Carbohydrates polymerization has been shown not to occur in MALDI-TOF; thus a different system of linkage than what was envisaged in structure (**III**) must be present.

In Figures 6–9 are shown the positive ion mode MALDI-TOF spectra of the four gallotannins analyzed. Sumach tannin (Fig. 6) extract is a polygallic tannin and its MALDI-TOF spectrum shows some major series of peaks exhibiting a mass increment of 152 Da (Fig. 6). This corresponds to what is known

TABLE II
Percentage Distribution of the Different Oligomers Present in Commercial Oak Tannin Extract (from MALDI-TOF Positive Ion Mode Spectra)

Da	Peak intensity	Relative abundance (%)	
570	17	3.1	1 Galloyl residue + 3 stripped glucose residues = trimer
576	100	18.2	Unknown
703	22	4.0	1Galloyl residue + 4 stripped glucose residues = tetramer
835	38	6.9	1 Galloyl residue + 5 stripped glucose residues = pentamer
959	67	12.2	Pentagalloyl glucose, monomer
967	45	8.2	1 Galloyl residue + 6 stripped glucose residues = hexamer
1092	55	10.0	1 Galloyl residue + 7 stripped glucose residues = heptamer
1232	52	9.4	1 Galloyl residue + 8 stripped glucose residues = octamer
1364	40	7.3	1 Galloyl residue + 9 stripped glucose residues = nonamer
1496	31	5.6	1 Galloyl residue + 10 stripped glucose residues = decamer
1628	27	4.9	1 Galloyl residue + 11 stripped glucose residues = undecamer
1761	17	3.1	1 Galloyl residue + 12 stripped glucose residues = dodecamer
1893	14	2.5	1 Galloyl residue + 13 stripped glucose residues = tridecamer
2025	12	2.1	1 Galloyl residue + 14 stripped glucose residues = tetradecamer
2157	8	1.5	1 Galloyl residue + 15 stripped glucose residues = pentadecamer
2289	6	1.1	1 Galloyl residue + 16 stripped glucose residues = hexadecamer

as regards the structure of this tannin. The repeat unit is structure (**IV**)



IV

However, some differences from this are evident in Figure 6. The first interpretable peak for this tannin is at 508 Da. This corresponds to a glucose to which are linked two gallic acid residues (23(Na^+) + 180 + 152 + 152 = 507 Da). Three different series of oligomer peaks are present.

The series of oligomers in the highest proportion starts with the 924-Da peak (Fig. 6). This oligomer corresponding to this peak is exclusively composed of a chain of five glucoses (23(Na^+) + 180 × 5 = 923 Da). The series continues with the peaks at 1076, 1229, 1381, 1532, 1684, 1836, and 1988 Da, thus adding one by one gallic acid 152 Da repeating units. It is not possible to assume that the peaks of the series represent linear chains of 1, 2, 3, 4, 5, 6, and 7 gallic acid residues attached to one of the glucoses only. It is more likely that the gallic acid residues are linked as single gallic residues or dimers or trimers to each of the five glucoses of the oligomer chain.

The closest interpretation of the 540-Da peak is based on two glucoses and a gallic acid residue (23 (Na^+) + 180 + 180 + 152 = 535 Da). It is not possible to determine how these are linked with each other.

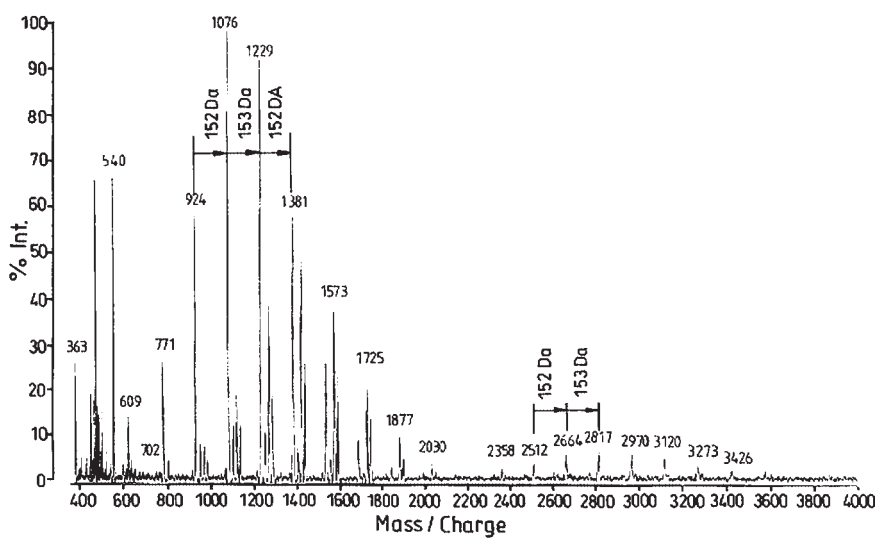


Figure 6 MALDI-TOF positive ion mode spectrum of water extracted sumach tannin extract in the 400–2600 Da mass range.

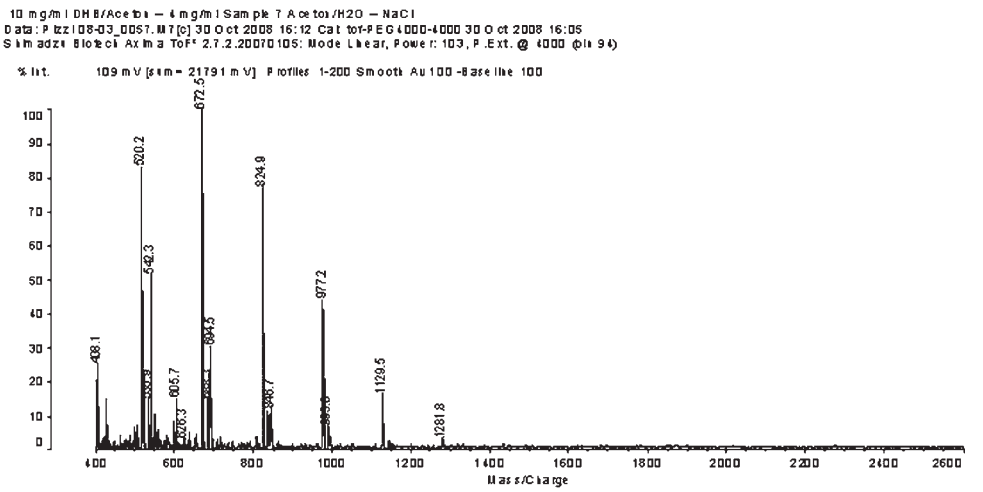


Figure 7 MALDI-TOF positive ion mode spectrum of solvent extracted tara tannin extract in the 400–2600 Da mass range.

The second series in the second highest proportion is the 508-, 965-, 1117-, 1269-, 1421-, 1573-, 1725-, 1877-, and 2030-Da series. The peaks correspond to a single glucose linked respectively to two gallic acid residues (508 Da), six gallic acid residues (965 Da), and so on up to a single glucose linked to 13 gallic acid residues (2030 Da). This clearly identifies them as polygallic acid chains of type (**IV**) because only one glucose is present in each of these oligomers. A chain of nine polygallic acid residues linked to a single glucose is the predominant oligomer in this series (at 1421 Da). It is interesting to note that in this series one passes from the gallic acid dimer linked to a glucose directly to the hexamer and higher oligomers, without any trimers, tretramers, or pentamers being present.

The third pattern, the least abundant, also presents a repeating unit of 152 Da. This is the series of peaks at 2358, 2512, 2664, 2817, 2970, 3120, 3273, and 3426 Da. These are higher oligomers, but notwithstanding the regular 152-Da increment are difficult to interpret. This series does not derive from any of the other two already described. Thus, the peak at 2358 does not derive from any peak of the other two series. The only correspondence is that it is formed by a glucose (or other sugar residue) oligomer of 13 repeating units ($23\ (Na^+) + 180 \times 13 = 2363$ Da). To these are attached seven gallic acid residues either as gallic acid oligomers or as monomers or dimers. The gallic acid residues are most likely linked as single gallic residues to each of the glucoses of the glucose oligomer chain, as for the first series.

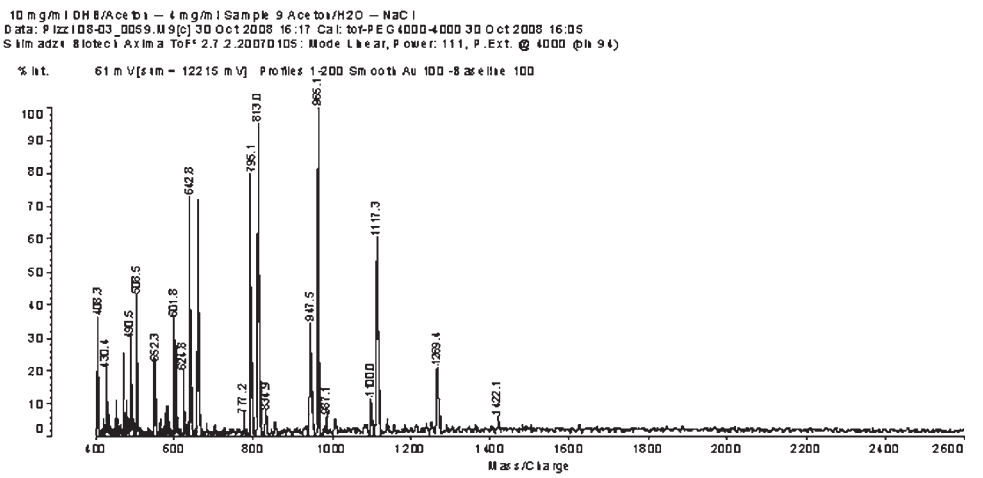


Figure 8 MALDI-TOF positive ion mode spectrum of solvent extracted turkey gall tannin extract in the 400–2600 Da mass range.

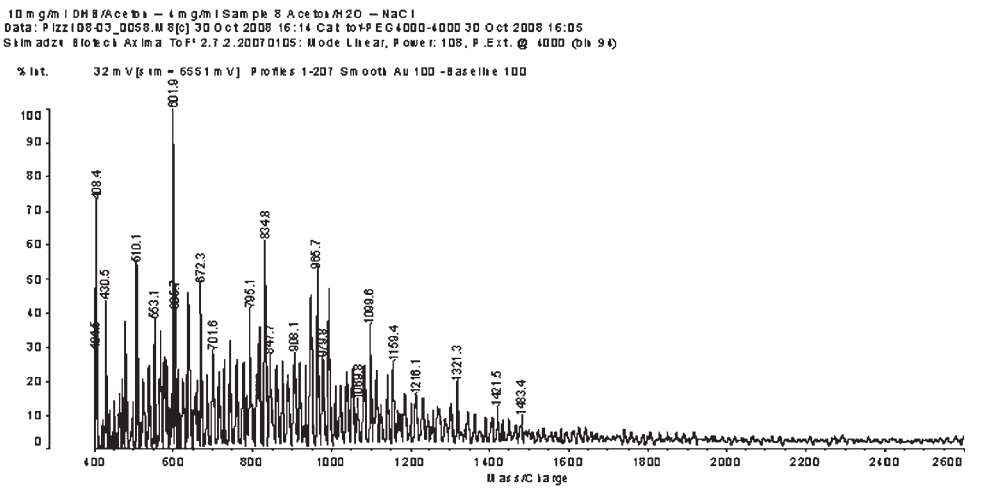
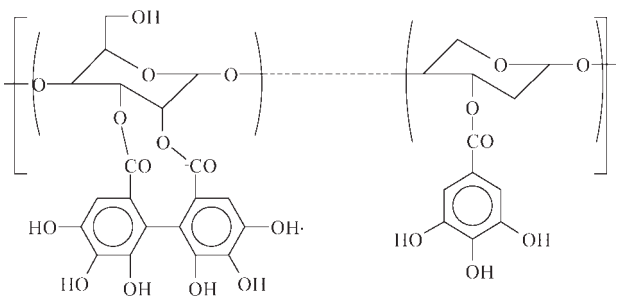


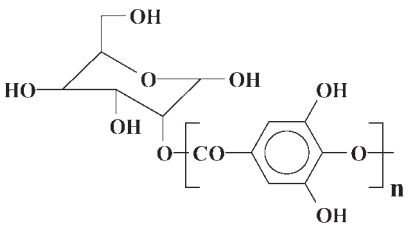
Figure 9 MALDI-TOF positive ion mode spectrum of solvent extracted Chinese gall tannin extract in the 400–2600 Da mass range.

Thus, according to the positive ions mode MALDI-TOF analysis, commercial sumach tannin extract is then composed of three types of oligomers. These are as follows:

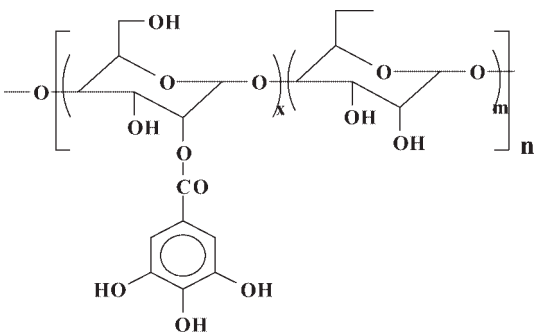
1. The pattern of peaks in highest relative proportion corresponding to structure (**V**) composed of five sugar residues and up to seven galloyl residues.
2. The pattern of peaks in the second highest relative proportion responding to structure (**VI**), with $n = 2$–13.
3. The third and minor series of peaks corresponding to structures of type (**VII**), where $m = 6$–13, $x = 13 - m$, $n = x + m$.



V



VI



VII

The relative abundance of the oligomers corresponding to these three patterns is shown in Table III.

The spectra of the solvent extracted tara and turkey gall tannins are shown in Figures 7 and 8. Solvent extraction has eliminated the carbohydrates in the extract; thus, the polymeric nature of these two tannins can be clearly observed. The repeating unit observed is 152 Da, as for sumach, and corresponds to the same basic structure (**IV**) indicated for sumach extract. In the case of tara tannin this is known to contain small amounts of ellagic acid and a much greater proportion of gallic acid.[6] The MALDI-TOF in Figure 7 confirms this finding. Thus, the second dominant peak at 520 Da (23 (Na^+) + (302 − 1H) + 152 + 44 = 520 Da) indicates an oligomer formed by one ellagic acid linked to one gallic acid, although the presence of an additional $-COO^-$ cannot be easily explained. The peaks at 672, 824, 977, 1129, 1281 Da indicate then a series of oligomers each formed by adding a gallic acid residue to the basic structure of the 520-Da peak. Thus, the number of gallic acid total residues indicates a series of polygallic trimers, tetramers, pentamers, hexamers, heptamers, and

TABLE III
Percentage Distribution of the Different Oligomers Present in Commercial Sumach Extract

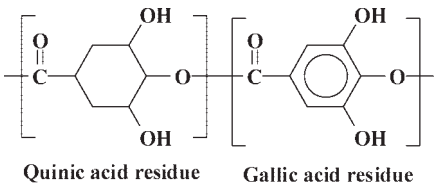
Da	Peak intensity	Relative abundance (%)	
540	67	9.45	Dimer
Pattern 1 (all glucose pentamers)			
924	60	8.45	Glucose pentamer
1076	100	14.1	Monogalloyl glucose pentamer
1229	92	12.9	Digalloyl glucose pentamer
1381	58	8.2	Trigalloyl glucose pentamer
1532	28	3.9	Tetragalloyl glucose pentamer
1684	10	1.4	Pentagalloyl glucose pentamer
1836	4	0.6	Hexagalloyl glucose pentamer
1988	1	0.15	Heptagalloyl glucose pentamer
	Total pattern 1	50.55	
Pattern 2			
508	66	9.2	Digalloyl glucose
965	8	1.1	Hexagalloyl glucose (hexamer)
1117	20	2.8	Heptagalloyl glucose (heptamer)
1269	40	5.6	Octagalloyl glucose (octamer)
1421	48	6.8	Nonagalloyl glucose (nonamer)
1573	40	5.6	Decagalloyl glucose (decamer)
1725	23	3.2	Undecagalloyl glucose (undecamer)
1877	10	1.4	Dodecagalloyl glucose (dodecamer)
2030	4	0.6	Tridecagalloyl glucose (tridecamer)
	Total pattern 2	35.4	
Pattern 3			
2358	2	0.3	Glucose tridecamer
2512	4	0.6	Monogalloyl glucose tridecamer
2664	5	0.7	Digalloyl glucose tridecamer
2817	7	1.0	Trigalloyl glucose tridecamer
2970	6	0.8	Tetragalloyl glucose tridecamer
3120	4	0.6	Pentagalloyl glucose tridecamer
3273	3	0.4	Hexagalloyl glucose tridecamer
3426	1	0.15	Heptagalloyl glucose tridecamer
	Total pattern 3	4.6	
Total		100	

octamers starting from the 520-Da peak. Their relative abundance is 26, 29, 23, 14.5, 6, and 1.5%, respectively. Oligomers smaller than trimers do not appear to occur.

A similar repeating unit pattern (152 Da) is observed for the positive ions mode MALDI-TOF of turkey gall tannin (Fig. 8). In this tannin the first interpretable peak is at 508 Da. This corresponds to a glucose to which are linked two gallic acid residues ($23(Na^+) + 180 + 152 + 152 = 507$ Da). It is not possible to know if the two gallic acid residues are separately linked to the glucose or if this is a digallic acid residue linked to the glucose. This latter appears to be the most probable configuration. It means that the 508-Da peak is a gallic acid dimer and the series of peaks at 661, 813, 965, 1117, 1269, and 1422 Da represent a series of gallic acid trimers, tetramers, pentamers, hexamers, heptamers, and octatamers, all attached to one glucose. The peak at 642 Da is the 661 peak minus an —OH, thus also a glucose + a gallic acid trimer. The same is valid for the peak at 795 Da, which is the 813-Da peak minus an —OH group, thus a glucose + a gallic acid tetramer. Due to their relative abundance, these two peaks (642 and 795 Da) must also be taken into account in calculating the relative proportion of the oligomers present in turkey gall tannin. Thus, from the spectra in Figure 8 the respective abundance is of 8% (digalloyl glucose, dimers), 26% (monoglucose galloyl trimers), 32% (monoglucose galloyl tetramers), 18% (monoglucose galloyl pentamers), 11% (monoglucose galloyl hexamers), 3.5% (monoglucose galloyl heptamers), and 1.3% (monoglucose galloyl octamers).

It must also be kept in mind that while the repeating unit molecular weight is 152 Da, residues of quinic acid linked in the chain can also be present. Quinic acid is known to be present and included in some manner in the gallic acid chains of tara tannin.[6–8] It appears to be linked in the chain in the

same manner as gallic acid (**III**), hence slightly differently than what was proposed in previous work.[7,8]

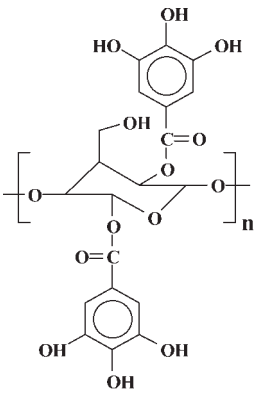


VIII

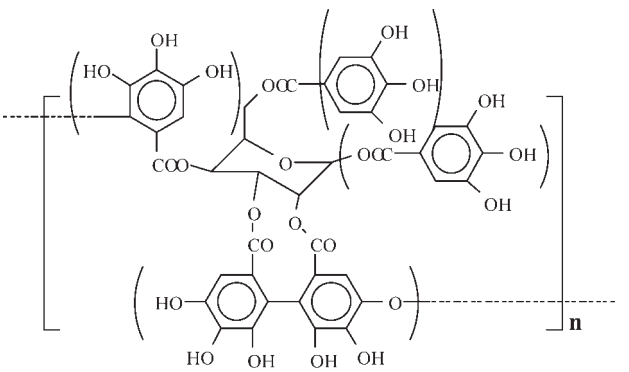
The same could apply to turkey gall tannin, although presence of quinic acid in the chain of this tannin has not been reported. Thus, this tannin must be still considered as a purely polygallic tannin linked to some glucose. This type of configuration is rather different from that proposed for residual quinic acid gallotannins (mainly tara) by previous authors.[7,8]

The repeating unit pattern is different, however, for the chinese gall tannin (Fig. 9). Here the repeating unit is 162 Da. Thus, the series of peaks that can be seen responding to this pattern is the series 510, 623, 834, 996, 1159, 1321, and 1483 Da. Such an unusual repeating unit can have two explanations. In one case this can correspond to a quinic acid that is linked to other different quinic acid chains through its hydroxy groups as reported in much earlier work for tara tannin,[7] and in a manner different to that reported for structure (**VIII**). Thus, quinic acid, where the $-OR_{1,2}$ are hydroxygroups, has a molecular weight of 168 Da; if one hydrogen is substituted with a gallic acid residue such as R_1, then the total weight is of 168 Da − 1H + 152 + 1H Da = 320 Da. This is close enough to 162 Da × 2 = 324 Da, the real repeating unit of the MALDI-TOF spectrum in Figure 9. Such an approach, however, cannot explain the multirepeating 162-Da pattern. This indicates that such an explanation is incorrect. Furthermore, the presence of quinic acid has never been reported in chinese gall tannins.

The second, more acceptable explanation is closer to what is already known on chinese gall tannin, namely, its composition of esters of gallic and digallic acid with glucose. Thus, two gallic acid residues attached to a glucose yield a repeating unit of [(180 − 1H Da) + 152 Da + (152 + 1 Da)] = 484 Da (similar to 162 Da × 3 = 486 Da). It cannot be determined if this is a digallic acid linked to glucose or two gallic acid residues each linked independently to glucose. This appears then to be the real repeating unit of the oligomers. The oligomeric structure of commercial chinese gall tannin is then rather different to what is observed for turkey gall tannin. The repeating unit appears to be either structure (**IX**) or most likely structure (**X**)



IX



X

where the parentheses indicate the manner in which the two (only) gallic acid residues can be attached to the glucose residue. Thus, the repeating units are formed by digalloyl glucose if the structure is interpreted on the basis of the MALDI-TOF-positive ion mode spectrum in Figure 9. It has been reported in the literature[7] that all the hydroxyl groups of chinese gall tannin are substituted by galloyl residues and thus that the repeating unit should be pentagalloyl glucose. The positive ion mode MALDI-TOF analysis of the commercial chinese gall extract does not support this structure as only two galloyl residues are clearly attached to the sugar residues, as in structure (**IX**). Whatever the mode of linkage to each other of the repeating units then, the peak at 510 Da is close enough to be the same as in turkey gall tannin, namely, a glucose to which are linked two gallic acid residues (23(Na^+) + 180 + 152 + 152 = 507 Da). The series that starts with the 510 Da (digalloyl glucose) continues with the 996 Da (digalloyl glucose dimer) and 1483 Da (digalloyl glucose trimer) oligomers. The other 162-Da repeating peaks belong to similar molecules in which alternatively a galloyl or two galloyl residues have been lost together with some part of the sugar residue. However, a better interpretation of this spectrum can be the same given for the two galloellagic tannins (chestnut and oak tannins).

Thus, in Figure 9, the 162-Da repeating unit can be interpreted as a glucose in which a single —OH has been lost. This indicates that in commercial chinese gall tannin a single monogalloyl, or digalloyl or trigalloyl residue, may be linked to each glucose of the main sugar skeletal chain, as already reported by earlier authors.[9] Thus, starting from the 510-Da peak (digalloyl glucose), the series that follows is formed by the 672-, 834-, 996-, 1159-Da peaks. This is, hence, a series of oligomers of up to glucose hexamers on which two galloyl residues are still linked onto a single sugar residue of the chain. To this series is superimposed the 510-Da (digalloylglucose), 996-Da (digalloyl glucose dimer), and 1483-Da (digalloyl glucose trimer) series. The 965-Da peak is likely to be a pentagalloyl glucose, although this is not at all dominant in this spectrum. This implies that monogalloyl, digalloyl, and trigalloyl groups were initially linked to this tannin. This is supported by the earliest previous work[9] on this tannin. Previous authors have shown that in chinese gall tannin and in sumach tannin the products isolated were essentially octa- and nonagalloylated glucose.[7] They estimated that the gallotannin contained a pentagalloylglucose nucleus to which some three or four additional galloyl groups were attached. The MALDI-TOF analysis presented here shows this not to be the case, or at least not the only case, and brings to the fore the skeletal polymeric nature of the carbohydrates of the galloyl glucose chains.

CONCLUSION

The structure of six commercial hydrolyzable tannins has been examined by MALDI-TOF MS. Their oligomeric structures and structure distribution have been defined and have in some cases been found to be similar to results reported by other authors for tannin extracted for analysis. However, in the commercially extracted tannins some marked differences from what already reported were noted. Degradation products of rather different structure than what was reported were present. Relevant pentagalloyl, trigalloyl, and digalloyl glucose monomers were observed in MALDI-TOF spectra of the galloellagic chestnut and oak tannin extracts and in one gallotannin, chinese gall tannin extract. In the positive ion mode spectra the most remarkable findings were that most of the galloyl residues of the galloyl glucose chains were stripped from the skeletal glucose chain carrying them. This allowed the observation of oligomers, in some cases up to 16 or 17 glucose units long, totally or almost totally stripped of galloyl residues, as their molecular weight came within the range of molecular masses more easily observed with MALDI MS. The stripped galloyl residues were found in the negative ion mode spectra, these residues being still present in the commercial extract. This indicated that a wide distribution up to very long galloylglucose chains exist in most commercial hydrolyzable tannin extracts. It also indicated that all these commercial tannin extracts are mainly composed of long pentagalloyl glucose chains in which some galloyl residues have been detached, giving the appearance of mixed di-, tri-, and pentagalloyl glucose repeating units being present in the same chain. This supported also the concept advanced previously by other evidence that such tannins may be present in the wood as an extended macromolecular chains network.

The pentagalloyl glucose units in tannin chains are considered to be linked through a galloyl residue of a unit to a galloyl residue of the unit that follows it and of the unit that precedes. However, the presence of long glucose chains in the positive ion mode MALDI-TOF spectra in the cases in which most or all of the galloyl residues have been stripped indicates that the manner in which the pentagalloyl glucose repeating unit is linked may be sugar residue to sugar residue. This is rather different from the normally accepted form of linkage of these tannins.

Commercial tara and turkey gall tannins have been shown to be mainly polygallic oligomers of up to eight gallic acid residues linked to each other in a chain. Commercial sumach extract revealed itself a more complex mixture of glucose oligomers up to 13 repeating units where to several but not all of the sugar repeating units is linked a galloyl or digalloyl residue.

References

1. Fengel, D.; Wegener, G. Wood, Chemistry, Ultrastructure, Reactions; Walther DeGruyter: Berlin, 1984.
2. Tang, H. R.; Hancock, R. A.; Covington, A. D. In Proceedings of the XXI IULTCS (International Union of Leather Trade Technologists and Chemists): Barcelona, Spain, 1991; p 1503.
3. Haslam, E. The Chemistry of Vegetable Tannins; Academic Press: London, 1966.
4. Roux, D. G. In Plant Polyphenols 1; Hemingway, R. W.; Laks, P. E., Eds.; Plenum: New York, 1992; pp 7–39.
5. Pasch, H.; Pizzi, A. J Appl Polym Sci 2002, 85, 429.
6. Garro-Galvez, J. M.; Riedl, B.; Conner, A. H. Holzforschung 1997, 51, 235.
7. Armitage, R.; Bayliss, G. S.; Gramshaw, J. W.; Haslam, E.; Haworth, R. D.; Jones, K.; Rogers, H. J.; Searle, T. J Chem Soc 1961, 1842.
8. Horler, H.; Nursten, H. E. J Chem Soc 1961, 3786.
9. Buziashvili, Sh.; Komissarenko, N. F.; Kovalev, I. P.; Gordienko, V. G.; Kolesnikov, D. G. Khimiya Prirodnykh Soedinenii 1973, 6, 789.

10. Karas, M.; Bachmann, D.; Bahr, U.; Hillenkamp, F. Int J Mass Spectrom Ion Phys 1987, 78, 53.
11. Bahr, U.; Deppe, A.; Karas, M.; Hillenkamp, F.; Giessmann, U. Anal Chem 1992, 64, 2866.
12. Danis, P. O.; Karr, D. E.; Mayer, F.; Holle, A.; Watson, C. H. Org Mass Spectrom 1992, 27, 843.
13. Danis, P. O.; Karr, D. E. Org Mass Spectrom 1993, 28, 923.
14. Ehring, H.; Karas, M.; Hillenkamp, F. Org Mass Spectrom 1992, 27, 472.
15. Pasch, H.; Resch, M. Polym Anal MALDI-MS GIT Fachz Lab 1996, 40, 90.
16. Pasch, H.; Gores, F. Polymer 1995, 36, 1999.
17. Pasch, H.; Pizzi, A.; Rode, K. Polymer 2001, 42, 7531.
18. Yoshida, T.; Hatano, T.; Ito, H.; Okuda, T. In Plant Polyphenols; Gross, G. G.; Hemingway, R. W.; Yoshida, T., Eds.; Plenum: New York, 1999; Vol. 2, pp 127–144.
19. Amakura, Y.; Yoshida, T. Chem Pharm Bull 1996, 44, 1293.