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Microstructure Elucidation of Polyflavonoid Tannins by MALDI-TOF-CID

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ABSTRACT: High molar mass wood tannin extracts are complex mixtures that are distributed in both molar mass and chemical composition. Condensed tannins from quebracho and mimosa woods were analyzed and compared with cacao tannins using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry. Although MALDI-TOF MS reveals the oligomer structure of the tannins, this method cannot distinguish between isomers with isobaric masses, and therefore, ambiguous structural assignments were made in a number of cases. To determine the actual microstructures present, MALDI-TOF collision-induced dissociation (CID) experiments were conducted. MALDI-TOF-CID enables monomer sequence determination, and positive assignments of isobaric structures can be made. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

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INTRODUCTION

Tannins are divided into two major groups, namely, condensed and hydrolysable tannins. Condensed tannins (also known as polyflavonoids or proanthocyanidins) are based on flavonoid units, which undergo condensation and polymerization reactions to form oligomers with varying degrees of polymerization (see Figure 1). In nature, these molecules are usually attached to their precursors, flavonoid analogs, carbohydrates, and traces of amino and imino acids.[1] Hydrolysable tannins differ from condensed tannins as they are derivatives of gallic acid and are usually esterified to a carbohydrate core, mainly glucose.[1–4] However, these tannins often occur as complex mixtures of simple phenols (e.g., pyrogallol), gallic and digallic acids as well as esters of sugars and other structures (e.g., three-dimensional networks) formed as a result of oxidative coupling and further esterification of the galloyl groups. The simplest of the hydrolysable tannins are gallotannins that are made up of polygalloyl esters of glucose such as pentgalloyl glucose.[4,5]

Condensed tannins can be found in the bark, stem, leaves, fruits, and other parts of various plants. Their molar masses typically range from 500 to 3000 Da, and they are used in various industrial applications such as leather tanning and wood adhesives.[2] Condensed tannin extracts are mostly composed of flavan-3-ol repeating units. The flavonoid repeating units are mainly linked to each other at C4-C6 in profisetinidins (resorcinol A-ring, gallocatechol B-ring) and prorobinetinidins (resorcinol A-ring, pyrogallol B-ring). On the other hand, the repeating units in procyanidins (phloroglucinol A-ring, catechol B-ring) and prodelphinidins (phloroglucinol A-ring, pyrogallol B-ring) are linked at C4-C8.[6]

In the analysis of both condensed and hydrolysable tannins, MALDI-TOF was used very successfully to determine the chemical composition of complex plant extracts.[7–13] The analysis of tannins using MALDI-TOF is a relatively new technique and provides additional information on the structure and molar mass distributions in a single experiment. Pasch et al.[10] applied MALDI-TOF to analyze polymeric tannins; the analysis of quebracho and mimosa extracts showed the difference in composition of these two similar wood extracts. Quebracho was shown to contain mainly profisetinidins that led to the formation of linear structures, whereas mimosa is predominantly composed of prorobinetinidins.[10] In the analysis of the chestnut extract, a hydrolysable tannin, the presence of vescalin/castalin and vescalagin/castalagin was conclusively determined.[9,12] MALDI-TOF has been successfully applied offline to analyze fractions obtained from a chromatographic separation; information obtained from this technique is very valuable as it can reach very high molar masses.[13–16] ESI-MS as an alternative method tends to form multiply charged molecules, whereas MALDI-TOF provides mostly singly charged molecules, thus making the

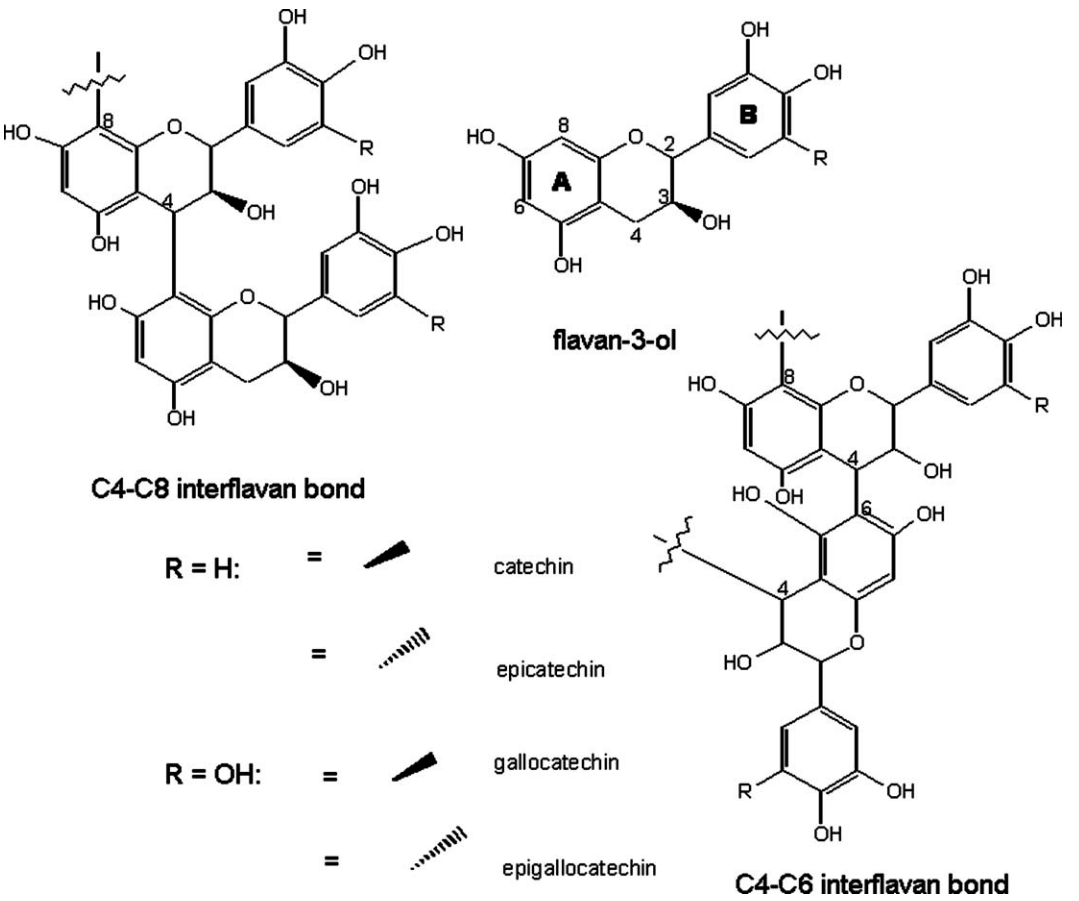

Figure 1. General structure of common condensed tannins.

spectra easier to interpret. One major problem of MALDI-TOF in particular for higher oligomers is that a given mass peak only provides the elemental composition, i.e., the number of A-, B-, or C-rings linked to each other. The sequence of these units in the macromolecule, however, cannot be obtained by MALDI-TOF.

A new promising technique for the analysis of tannin monomer sequences is post source decay (PSD) fragmentation, whereby a specific ion is selected from a MALDI-TOF spectrum and is subjected to higher laser intensities.[14,17] The high laser intensity results in fragmentation of the 'parent or precursor' ion and these fragments are detected. Ion dissociation is induced by the excess of internal energy that ions gain from the laser during the ionisation step. When the ions fragment in the field-free region, the process is termed PSD.[14,18] Behrens et al.[14] were the first to show the applicability of this method to tannins and condensed tannins from lime and spruce. Another method that performs a similar type of analysis is collision-induced dissociation (CID), whereby the precursor ion is selected from the first TOF analyzer and introduced into a collision cell, whereby it collides with inert gas molecules. The fragments formed are then reflected and analyzed in the second TOF analyser.[18–20] Although this technique has not been used for the analysis of tannins, it has been shown to be applicable to biopolymers as well as synthetic polymers and thus shows great promise for analysis of tannins.[18,19] Recently, ion trap mass spectrometry has been used for the analysis of tannins. Corke and co-workers[21,22] identified phenolic antioxidants in Chinese roses and galls, whereas Zhang and Lin[7] analyzed condensed tannins in Pacific oak leaves.

The aim of this study is to elucidate the microstructure of various wood tannin extracts by MALDI-TOF CID. The focus will be on the analysis of condensed tannins. It shall be

Table I. Sample List with Description and Tannin Type

Sample	Description	Type of tannin
1	Cacao extract	Condensed tannin
2	Water-extracted quebracho tannin	Condensed tannin
3	Water-extracted (bisulfited) quebracho tannin	Condensed tannin
4	Modified quebracho tannin (product of reaction with maleic anhydride and NaOH)	Condensed tannin
5	Solvent-extracted quebracho tannin	Condensed tannin
6	Water-extracted mimosa tannin (bisulfited)	Condensed tannin

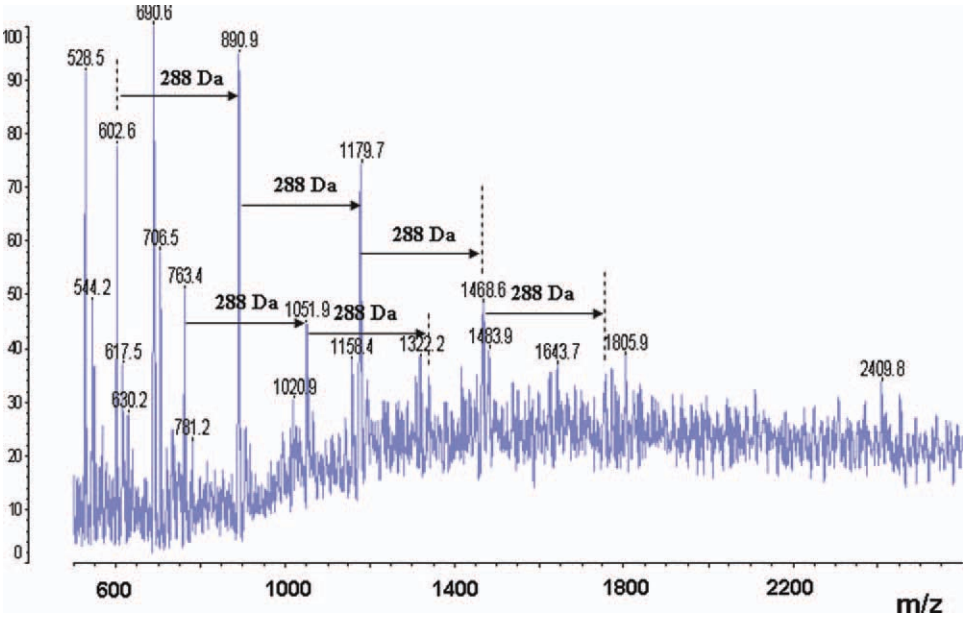

Figure 2. MALDI-TOF spectrum of cacao tannin extract (sample 1). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

demonstrated that MALDI-TOF CID is a unique method to determine the sequence of the different flavonoid units in the tannin molecules.

EXPERIMENTAL

Materials

Sample Preparation. The samples were prepared using a typical industrial extraction process. They were supplied by the Wattle Industry Marketing Committee being produced by NTE at Hermansburg, Natal, South Africa. The extraction for all but the mimosa tannin was conducted exclusively with hot water, by countercurrent extraction at 90°C to a guaranteed tannins content (thus from trimers upwards) of 74% and total phenolics of between 80 and 82%, the rest being mainly polysaccharides, sugars, some small amount of amino acids, and 4–6% water. No chemicals were used in the extraction. For the mimosa tannin extraction, hot water at 70°C and 1–2% sodium bisulfite was used. In this case, a very small amount of sulfonic acid groups may be attached in the C2 position. The general structure of the material, however, does not change.

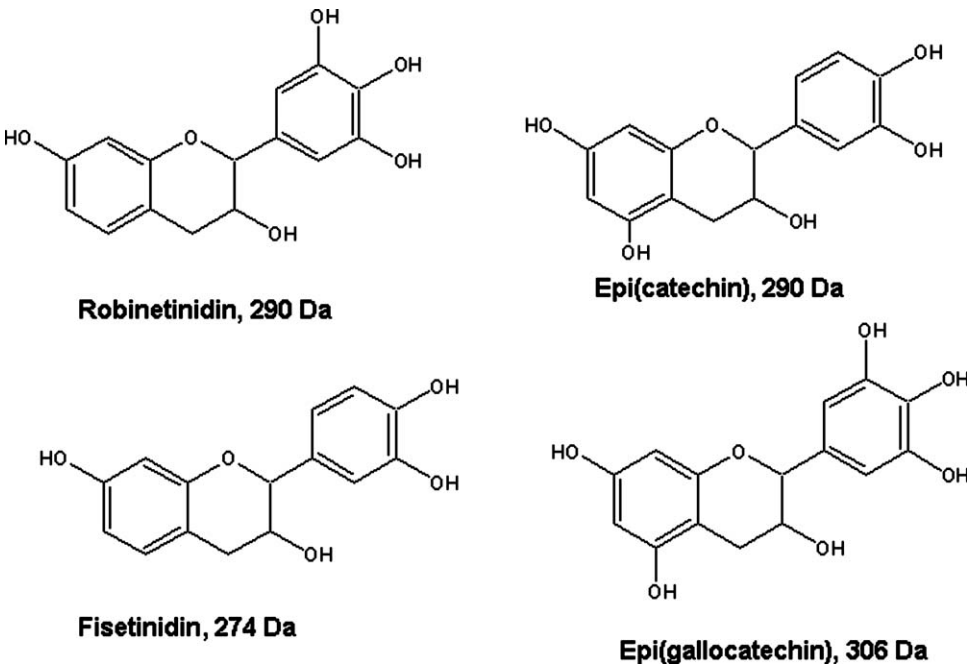

Figure 3. Building blocks present in the condensed tannin extracts with their molar masses indicated (H-end groups included in the molar masses).

Table II. Calculated and Observed Peak Masses of the Solvent-Extracted Cacao Tannin (Sample 1), the Predominant Repeat Unit Is 288 Da, Data from MALDI-TOF

	Linear positive			Unit type[a]		
Oligomer	Calculated M + Na^+	Observed M + Na^+		A	B	C
Dimer	569.6	567		2	—	—
	601.6	602[b]		1	—	1
			or	—	2	—
	617.6	618		—	1	1
Trimer	889.9	891[b]		1	1	1
			or	—	3	—
	905.9	906		—	2	1
			or	1	—	2
Tetramer	1178.2	1178[b]		—	4	—
			or	2	—	2
	1194.2	1194		1	1	2
			or	—	3	1
Pentamer	1466.5	1466[b]		—	5	—
			or	1	3	1
	1482.5	1483		—	4	1
			or	2	—	3
Hexamer	1754.8	1754		—	6	—
			or	2	2	2
Heptamer	2059.1	2057		—	6	1
			or	1	4	2
Octamer	2331.4	2329		2	4	2
			or	1	6	1

[a]Unit molar masses: A, 272 Da; B, 288 Da; and C, 304 Da, [b]Represents the dominant oligomer peak.

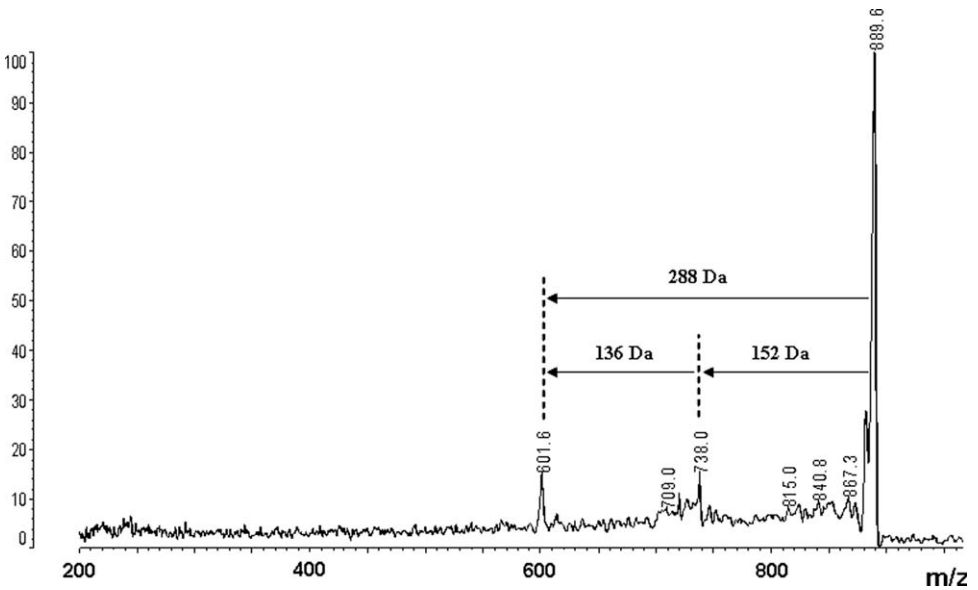

Figure 4. CID fragmentation spectrum of the trimer with m/z 889 from the cacao tannin extract (sample 1).

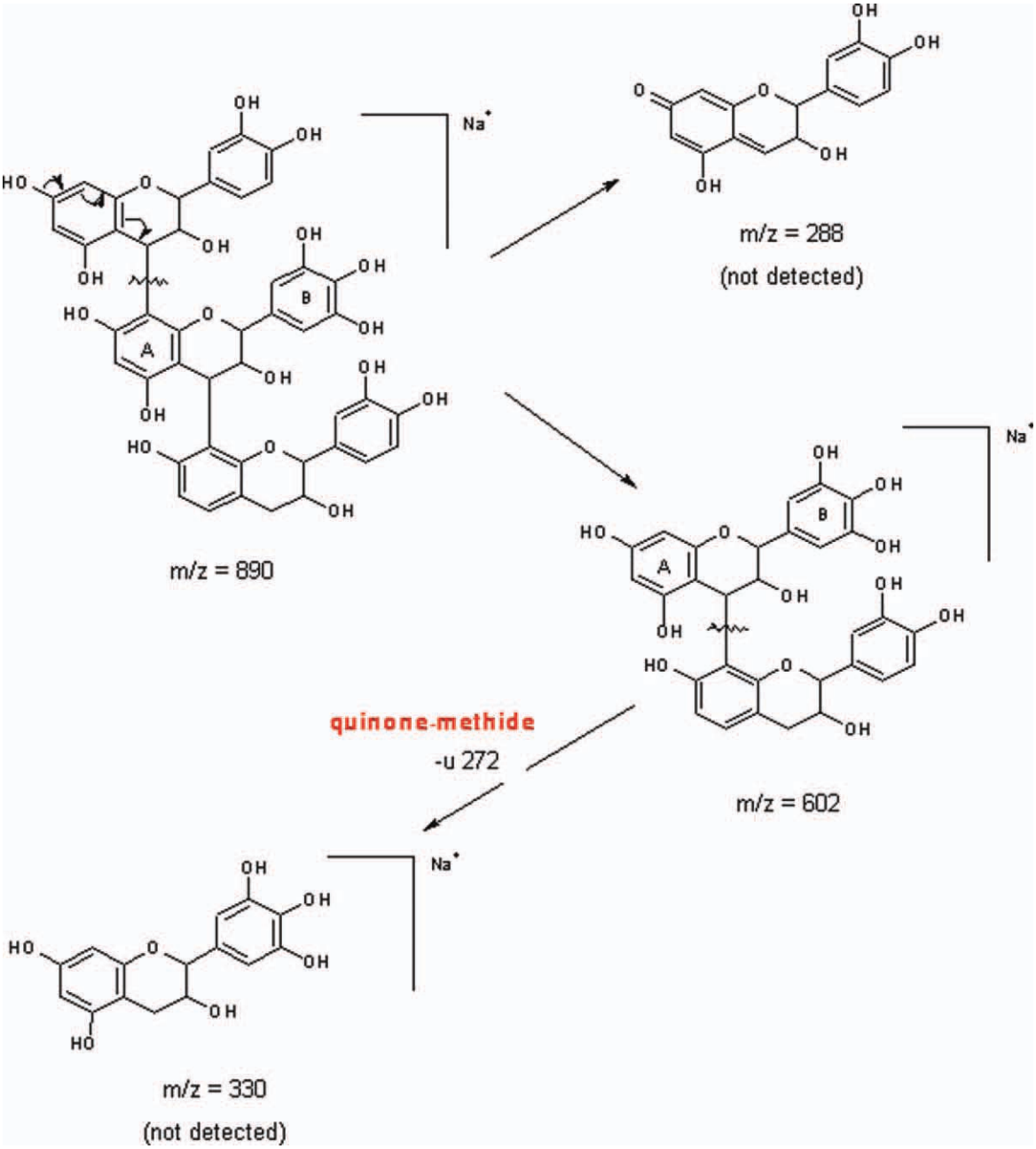

Figure 5. Suggested fragmentation pattern of the cacao tannin trimer with m/z 889.6. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

MALDI-TOF and MALDI-TOF CID Analysis

MALDI-TOF-CID experiments were performed on an Axima-TOF^2 spectrometer (Shimadzu Biotech, Manchester, UK), equipped with a nitrogen laser (337 nm), an ion gate for the selection of precursor ions, and a collision cell. The windows for separation of precursor ions were approximately 4 Da. Argon has been used as the collision gas. The pressure within the collision cell was 8×10^{-6} mbar. The pulsed extraction ion source accelerated the ions to a kinetic energy of 20 keV. All data have been obtained in positive ion linear mode applying the accumulation of 441 scans per spectrum. The calibration of the linear mode as well as the reflectron mode for CID analysis was done using PEG in mass range up to 2000 Da. The accuracy of the product ion calibration is ~0.5 Da. The mass calibration was conducted based on the average masses. Compared with the monoisotopic masses and depending on the accuracy of the calibration, mass differences of 1–2 Da may be observed. This inaccuracy, however, does not disturb the peak assignment. Higher resolution can be obtained by using the reflectron mode. Examples for more accurate MALDI-TOF work are given in Ref. 23.

The samples were dissolved in acetone/water 50/50 (% v/v) at a concentration of 4 mg/mL. The sample solutions were mixed with a 10 mg/mL solution of the matrix in the same solvent.

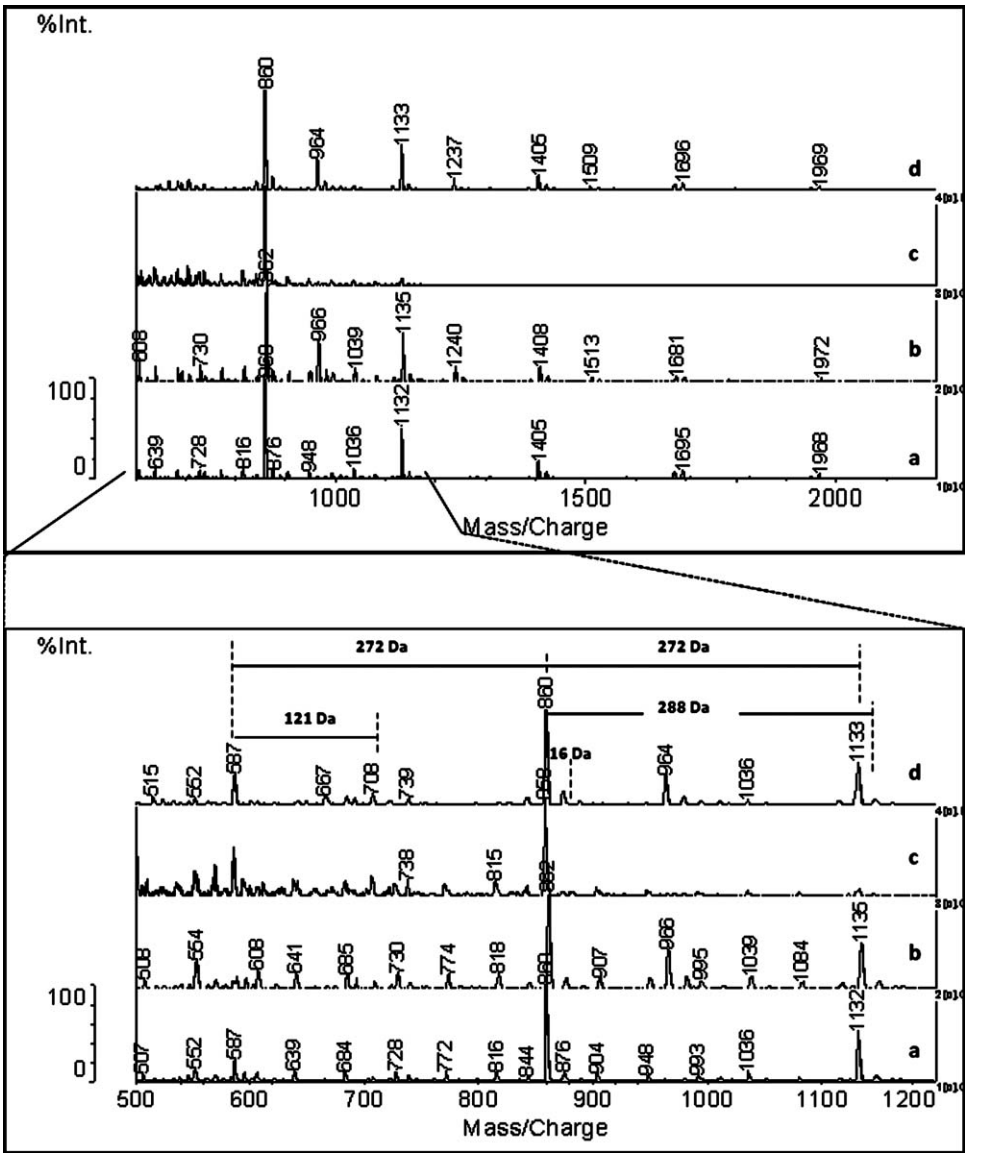

Figure 6. MALDI-TOF spectra of quebracho tannins (a) water extracted (sample 2), (b) bisulphited water extracted (sample 3), (c) modified (sample 4), and (d) solvent extracted (sample 5).

2,5-dihydroxy benzoic acid was used as the matrix. NaCl was added as the salt to enhance ion formation. The sample and matrix were then combined at ratios of 1 : 1. 5 μL of the resulting solution was spotted on a 384-well MALDI-TOF plate, followed by evaporation of the solvent at ambient temperature without any assistance. The MALDI-TOF target was then analyzed to give the resulting spectra. In all cases, for the MALDI as well as for the MALDI-CID experiments, a very good reproducibility of the spectra was obtained both regarding the ion peaks and their positions.

RESULTS AND DISCUSSION

In this study, quebracho, mimosa, and cacao tannins shall be investigated as typical examples for condensed tannins. They are extracted in large amounts industrially, and the cacao extract has been extensively studied in the field of food science (Table I).[1,12,24] Quebracho and mimosa tannins are known to be composed of profisetinidin and prorobinetinidin oligomers. The cacao tannin consists of the simpler counterparts, procyanidin.

Cacao Tannin Extract

The MALDI-TOF spectrum of the cacao extract (sample 1) shows a major oligomer distribution where the peaks are separated by masses of 288 Da, which in principle may be due to catechin or profisetinidin repeating units (B-type structure; see Figure 2).

The cacao tannin is known to be a procyanidins, and therefore, the B-type structure represents a catechin unit. Other possible units may be A and B rings having different numbers of OH-groups. In principle, repeating units with masses of 272, 288, and 304 Da are possible, which are defined as A, B, and C units, respectively. Typical building blocks for these repeating units are shown in Figure 3.

Table III. Calculated and Observed Masses of the Water-Extracted Quebracho Tannin (Sample 2) by MALDI-TOF

Oligomer	Calculated M + Na^+	Observed M + Na^+ Linear positive	Unit type[a] A	Unit type[a] B
Dimer	585.6	587[b]	1	1
	601.6	602	—	2
Trimer	841.9	842	3	—
	857.9	859[b]	2	1
	873.9	875	1	2
Tetramer	1114.2	1116	4	—
	1130.2	1132[b]	3	1
	1146.2	1148	2	2
Pentamer	1386.5	1389	5	—
	1402.5	1405[b]	4	1
	1418.5	1422	3	2
	1434.5	1438	2	3
Hexamer	1658.8	1661	6	—
	1674.8	1677[b]	5	1
	1690.8	1695[b]	4	2
	1706.8	1711	3	3
Heptamer	1947.1	1950	6	1
	1963.1	1967[b]	5	2
	1979.1	1983	4	3
Octamer	2219.4	2222	7	1
	2235.4	2239[b]	6	2
	2247.4	2250	5	3
Nonamer	2507.7	2512	7	2
		2529	6	3
		2542	5	4
Decamer	2780.0	2784	8	2

The predominant repeat unit is 272 Da.
[a]Unit molar masses: A, 272 Da; B, 288 Da; C, 304 Da, [b]Represents the dominant oligomer peak.

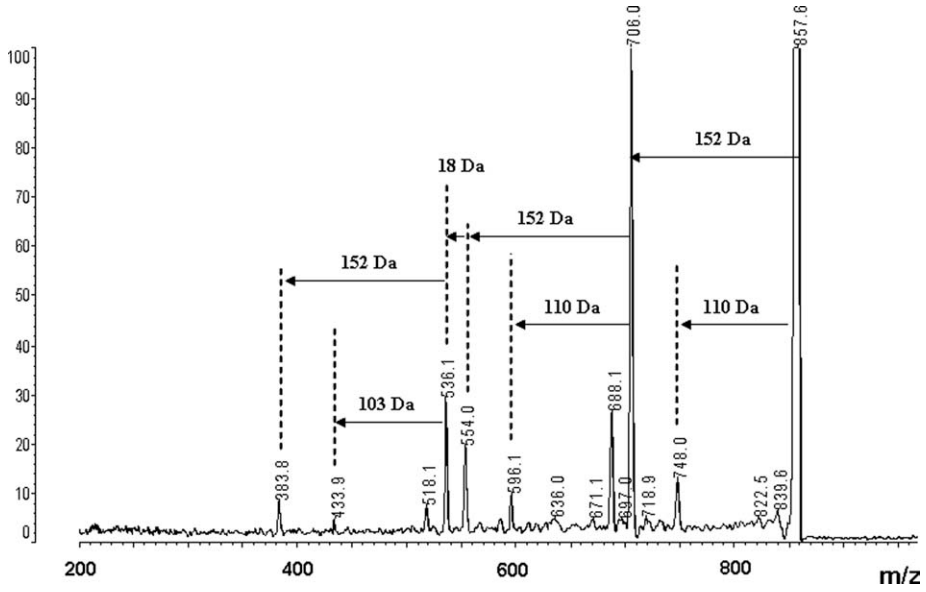

Figure 7. CID fragmentation spectrum of the trimer with the mass of m/z 857 from the solvent extracted quebracho tannin (sample 5).

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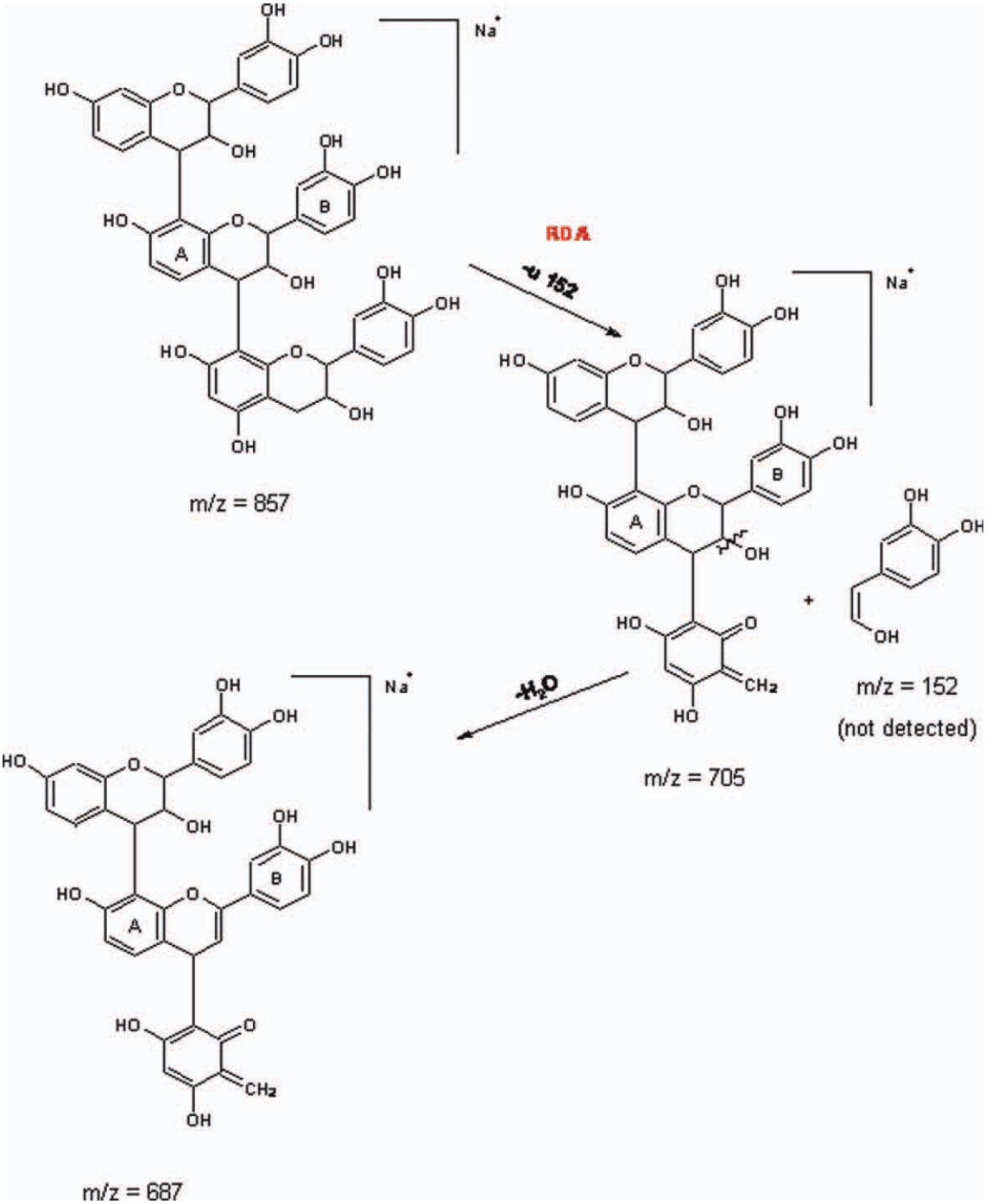

Figure 8. Suggested fragmentation pattern of the quebracho tannin using the RDA fission. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Assuming that ionization takes place through the attachment of a Na^+ cation, the theoretical oligomer masses can be calculated as follows: M + Na^+ = 23 (Na) + 2 (2 × H-end groups) + 272A + 288B + 304C. The peak assignment for the cacao spectrum is shown in Table II.

As can be seen from Table II, the same peak mass can be assigned to different combinations of A, B, and C units and, therefore, the structure assignments may be ambiguous and direct determination of the content of a specific repeating unit is not possible. This is, indeed, a major problem when it comes to oligomeric tannin analysis.

Fragmentation of the oligomers using CID experiments can assist in providing more detailed information with regard to the specific composition of a given oligomer. In the case of the cacao tannin extract, the precursor ion at m/z 889 was selected for fragmentation. This mass may arise from three B-type units or a combination of one A, B, and C unit each. The fragment spectrum of this mother ion is presented in Figure 4.

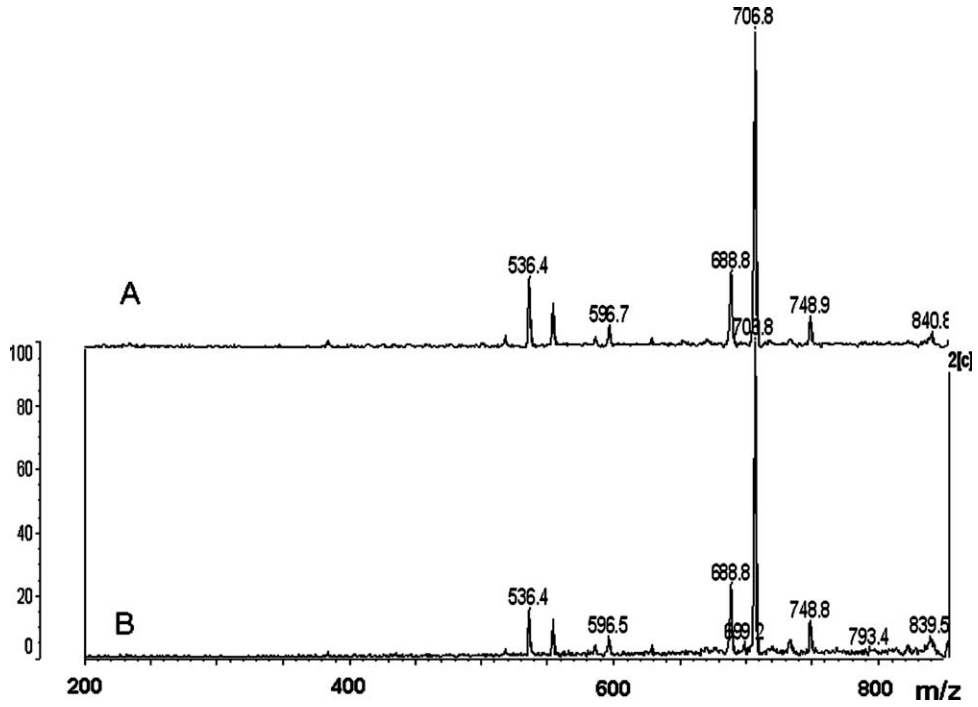

Figure 9. PSD fragmentation spectrum of the trimer with the mass of m/z 857 from the solvent-extracted quebracho tannin (sample 5), (A) with laser power of 120 and (B) laser power of 110.

The fragmentation results in a relatively simple pattern with a mass peak at m/z 601.6 having a mass difference of 288 Da towards the precursor ion. This fragment ion is the lowest mass ion formed and no smaller fragments are observed. There are various mechanisms that have been suggested to occur during CID.[14,25,26] In a Retro-Diels-Alder (RDA) fission fragments with masses of 152 or 138 Da can be formed as has been documented for ESI with atmospheric pressure chemical ionization. Another possible mechanism is related to a mass loss of 288 Da. The fragmentation of the present precursor ion based on this quinonemethide mechanism is shown in Figure 5.

The loss of a fragment of 152 Da from the precursor ion and the formation of the peak at m/z 738.0 correspond to the loss of a single B ring from the terminal unit. This fragment can be assigned to a galloyl unit. Accordingly, the original trimer must have contained a C-type (gallocatechin) structure. Unfortunately, the peaks at m/z 867.3, 840.8, and 815.0 could not be assigned. Although, based on the present result the monomer sequence in the trimer could not be determined, the fragmentation pattern unmistakably indicates that the trimer consists of a catechin, a fisetinidin, and a gallocatechin monomer unit.

Quebracho Tannin Extracts

For quebracho, water- and solvent-extracted samples were analyzed; the most detailed analysis being conducted on the solvent extracted sample 5. For all the quebracho extracts, the main signals are separated by 272 Da, this mass corresponding to a profisetinidin monomer unit (see Figure 6 for peak series at m/z 860-1132-1405). The water extracted quebracho tannin (sample 2) is shown in Figure 6(A). This spectrum shows an additional peak series, which also has the same repeat unit as the main series, see series at m/z 964–1253. This set of peaks might represent oligomers that have undergone a degradation reaction. This occurs via an elimination reaction favoring the removal of the A ring and the C4 from the heterocycle of the proanthocyanidin structure.[10] In addition, there are other significant peaks that are observed in the MALDI-TOF spectrum, which have a 16 Da mass difference from the peaks forming the major series. These peaks indicate the presence of other types of structures such as robinetinidol, which consists of an additional hydroxyl group on the B ring forming the B-type structures.[10,27] Although in this case the same mass of 288 Da given by this monomer can also be attributed to catechin, the quebracho tannin has been shown to consist of profisetinidins and probinetinidins.[6] The assignment of the mass peaks is given in Table III. The C-type structure (prodelphinidins) was excluded from the calculation because these structures are known not to be present in this extract.[10,26] Oligomers up to nonamers were detected in the water-extracted quebracho tannin. The assignment that is presented for sample 2 also applies for the other samples given in Figure 6.

The different quebracho extracts exhibit similar patterns in their main content; however, in the analysis of other extracts either modified or extracted by other methods, minor differences in their MALDI data were observed. For instance, the peaks observed at m/z 772 and 904 indicate an acetylated dimer and trimer, respectively, and the highest intensity is detected in the extract that has undergone acid/base treatment. The acetylation of some of the –OH groups occurs as a result of this process and can be detected in small amounts.[10]

The main constituent of quebracho tannin is the profisetinidin repeating unit regardless of the method of extraction. The

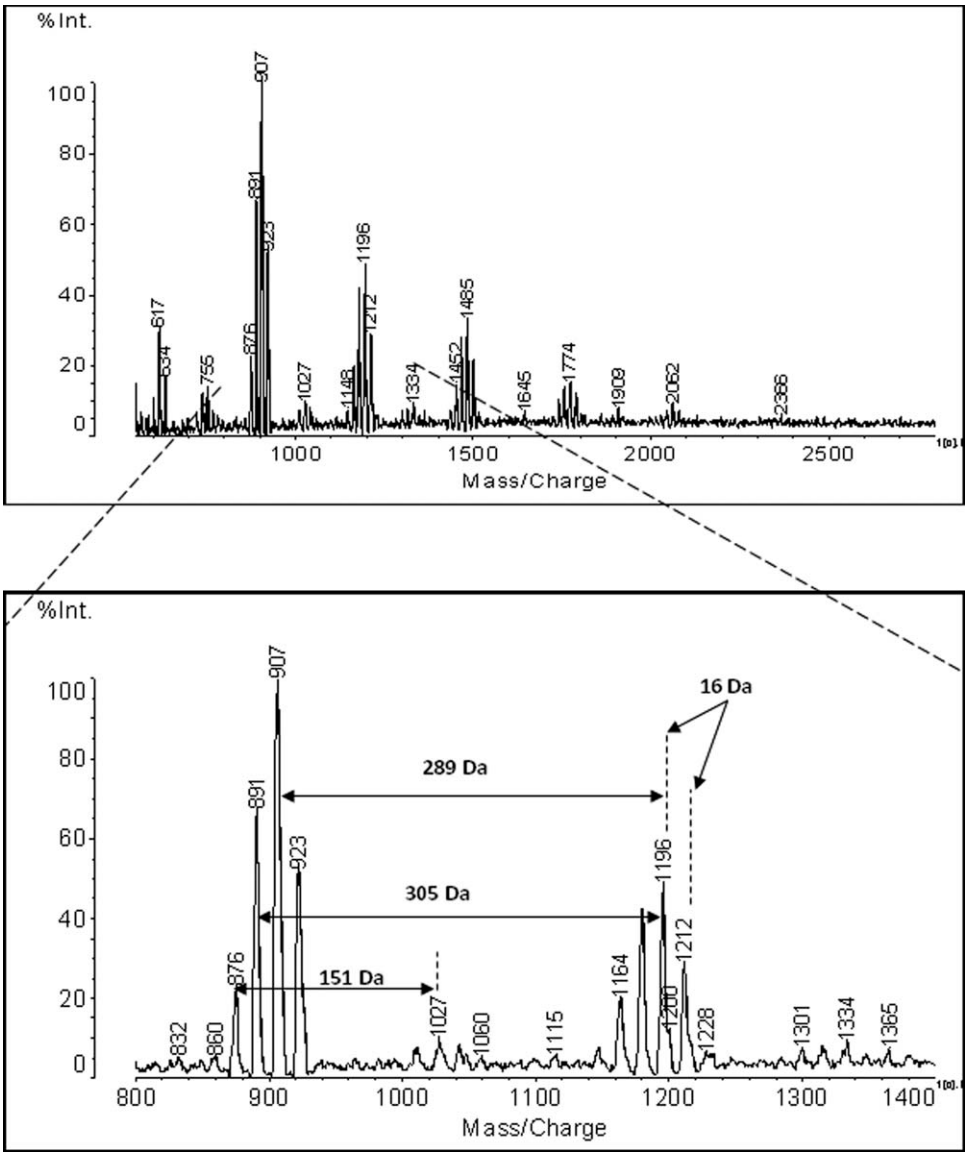

Figure 10. MALDI-TOF spectrum of bisulphited water extracted mimosa tannin (sample 6).

method of extraction and modification affects mainly the degree of polymerization and to a smaller degree the chemical composition and end-group functionality.

For further structural elucidation, the trimer of the solvent extracted quebracho tannin at an m/z 857 was selected as the precursor for the CID experiment. The MALDI-TOF CID spectrum for this oligomer is presented in Figure 7. The most abundant mass increment in this case is 152 Da. This is different from the pattern observed for the cacao tannin; therefore, the structure of the two trimers must be significantly different. The same fragmentation pathway was observed in ESI-MS and was shown to be to a Retro-Diels Alder fission tending to remove the B ring from the oligomer chain.[24,25] The RDA fission of the m/z 857 ion forms the fragment at m/z 706, which then looses a water molecule from the C3/C4 of the heterocycle to form the fragment ion at m/z 688. The loss of 152 Da occurs until smaller fragments are formed. In addition, the loss of a water molecule always seems to occur simultaneously with this mechanism. The peaks at m/z 748 and 596 are also formed due to the RDA reaction; however, these must be secondary products. The m/z 748 ion for example is 109 Da shifted from the precursor ion. This mass can only be explained by the loss of an A ring from a fisetinidin monomer (A-type unit). The mechanism of this reaction has not been reported yet. If this hypothesis is correct, then it means that the structure of this oligomer can be definitively described as A-type + A-type + B-type unit, in this order. This conclusion is reached because the loss of 152 Da can only occur at the terminus of the molecule. The 121 Da mass difference observed between the peaks at m/z 554 and 433 could be due loss of the A-ring on the B-type unit (Figure 7).

The mode of fragmentation whereby the interflavonoid bond is cleaved as in the case of the cacao tannin is not observed for the quebracho extract. Unlike in the case of ESI-MS whereby both modes are observed, MALDI-TOF-CID seems to prefer a

Table IV. Calculated and Observed Masses of the Bisulphited Water-Extracted Mimosa Tannin (Sample 6) Obtained by MALDI-TOF

Oligomer	Calculated $M + Na^+$	Observed $M + Na^+$ Linear positive		Unit type[a] A	B	C
Dimer	569.6	569		2	—	—
	585.6	586		1	1	—
	601.6	602		1	—	1
			or	—	2	—
	617.6	617[b]		—	1	1
	633.6	633		—	—	2
Trimer	857.9	860		2	1	—
	873.9	875		1	2	—
			or	2	—	1
	889.9	891		1	1	1
			or	—	3	—
	905.9	907[b]		—	2	1
	921.9	923		—	1	2
Tetramer	1114.2	1115		4	—	—
	1146.2	1148		2	2	—
	1162.2	1164		1	3	—
	1178.2	1180		2	—	2
			or	—	4	—
	1194.2	1196[b]		—	3	1
	1210.2	1212		—	2	2
	1226.2	1228		—	1	3
	1242.2	1247		—	—	4
Pentamer	1418.5	1421		4	—	1
	1434.5	1438		2	3	—
	1450.5	1452		1	4	—
			or	3	—	2
	1466.5	1469		1	3	1
			or	—	5	—
	1482.5	1485[b]		2	—	3
	1498.5	1501		1	1	3
			or	—	3	2
	1514.5	1516		1	—	4
			or	—	2	3

The predominant repeat unit is 288 Da, corresponding to a fisetinidin monomer (data only shown up to pentamers)
[b]Dominant oligomer, [a]Unit molar masses: A, 272 Da; B, 288 Da; C, 304 Da.

method depending on structure, see a suggested fragmentation pattern in Figure 8.

The peculiar behavior of MALDI-TOF in fragmenting molecules was investigated further by analyzing the quebracho tannins with a softer fragmentation, namely PSD.[28] In this experiment, the collision gas was switched off and the analysis was carried out at differing laser intensities. As can be seen in the spectrum presented in Figure 9, the fragmentation pattern is the same; however, in CID, the intensity of the peaks is higher and smaller fragments are able to form. Another point is that when the laser intensity increases so does the intensity of the peaks observed in the spectra. It is worth noting that the laser intensities mentioned here are the same ones normally used for tannin analysis, meaning that this form of fragmentation is inherent in the technique as well. The laser intensity on the other hand cannot be lowered in the analysis, as this gives poorly resolved spectra, and in some case, the analyte molecules do not receive sufficient energy to fly. This discovery means that care should be taken when assigning structures to reflectron-mode MALDI-TOF spectra, especially in the lower molar mass range where the fragments are located.

Table V. Calculated and Observed Masses of the Bisulphited Water-Extracted Mimosa Tannin (Sample 6) Obtained by MALDI-TOF

Oligomer	No. galloyl units	No. C-type units	Calculated $M + Na^+$	Observed $M + Na^+$ (positive linear)
Trimer	0	0	873.9	875
	1	0	1025.9	1027
	0	1	889.9	891
	1	1	1057.9	1059
Tetramer	0	0	1162.2	1164
	1	0	1314.2	1315
	1	1	1346.2	1348
	1	2	1362.2	1365
	1	3	1378.2	1379
Pentamer	0	1	1418.5	1420
	1	2	1602.5	1605
Heptamer	0	2	2043.1	2045
	1	2	2195.1	2197

The data shown indicate the number of galloyl units included in an oligomer.

Mimosa Tannin Extract

In the mimosa extract, the difference between the major peaks is 288 Da. This results in this extract having the highest content of angular structures of the tannin extracts discussed. However, this is not the only major distribution, as can be seen from the spectra in Figure 10 where the structure contains various isomers in higher intensity than the quebracho and cacao extracts.

The mimosa tannin is known not to contain procyanidins but prorobinetidin.[17] Therefore, its second major constituent is prodelphinidin, and this is evident from the MALDI-TOF spectra. Of the three types of tannins discussed, this is the only one that consists of angular trimers at m/z 1211 going up to pentamers as first shown by Oo et al.[12] When a bisulfited and a water-extracted mimosa tannin are compared, the MALDI data show that there is no major difference. However, the bisulfited sample gave higher signal intensities in MALDI analysis, and therefore, the discussion will focus on this extract. The peak assignments for this sample are shown in Table IV.

The relative similarity in structure of both mimosa extracts also confirms the observations made by Pasch et al. that the "linear" structure of the quebracho tannin is more susceptible to degradation than the relatively "branched" structure, and this is the reason for the minor structural change after sulfitation of this extract.[12,17] In the mimosa extract, an additional distribution, which is 152 Da from the major peaks, is present; as previously mentioned, this mass represents the presence of galloylated structures in the extract. An example is 1315 − 1164 Da = 152 Da; the peak at m/z 1164 is a tetramer and on attachment of a galloyl unit gives the mass at m/z 1315. The data for the galloylated structures are shown in Table V. To calculate the theoretical mass, the following equation was used: $M + Na^+ = 23$ (Na) + 152 (galloyl, end group) + 272 A + 288 B + 304 C. Although this seems to be the most likely structure, one may consider the possibility of some low energy fragmentations, which are possible even under mild conditions. It is very difficult to form galloylated structures because a massive rearrangement needs to occur for these type structures to be present. There is another possibility though: both the mimosa and quebracho extracts contain phloroglucinol A rings and the heterocycle can open-up and

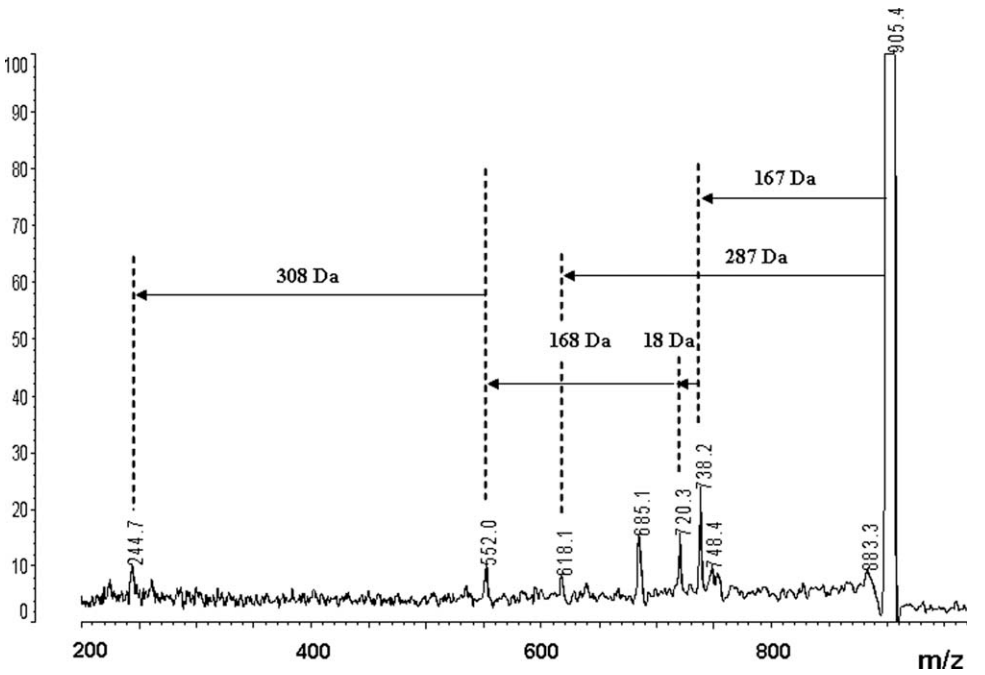

Figure 11. CID fragmentation spectrum of the trimer with the mass of m/z 905 from the bisulfited water-extracted mimosa tannin (sample 6).

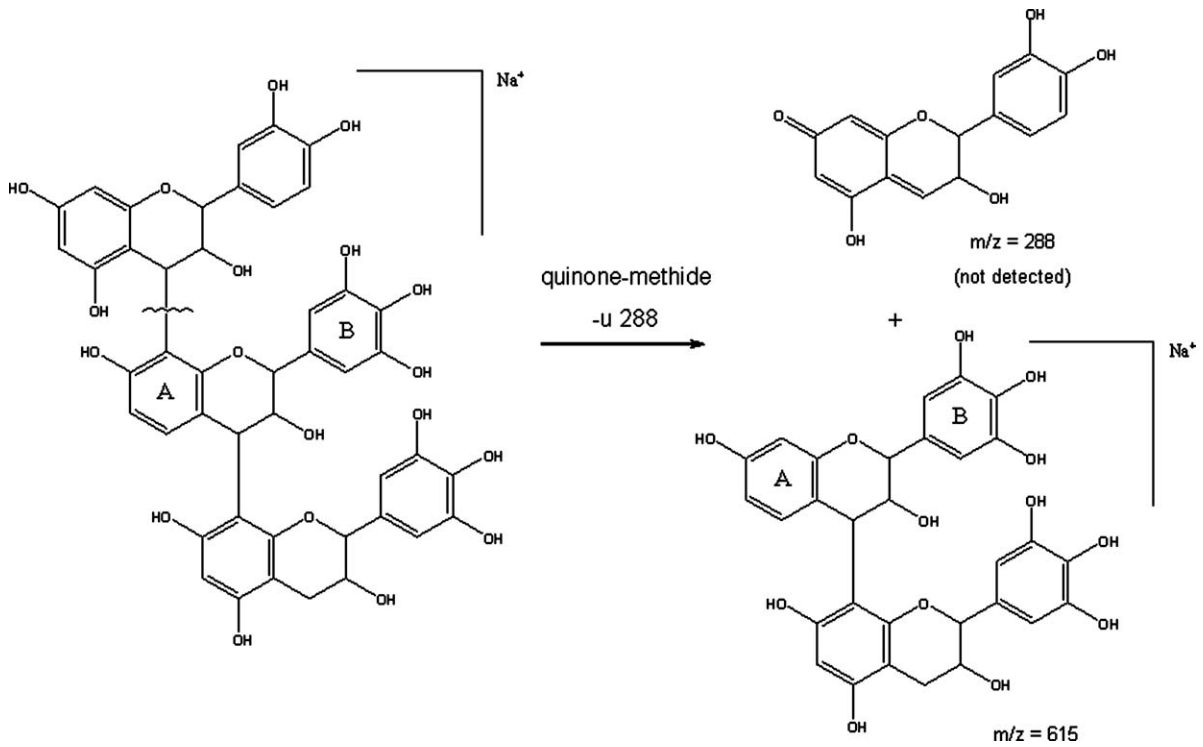

Figure 12. Suggested CID fragmentation pattern of the mimosa tannin trimer making use of the quinone-methide mechanism (Adapted from Rohr et al.[25])

cause the lower-end phloroglucinol ring to be released and this will have the same mass as a gallic acid residue.

Mimosa extracts are less susceptible to degradation and both the method of extraction and modifications have a lesser influence on the structure of the tannin. The bisulfitation reaction replaces the –OH groups on the heterocyclic structure and thus this causes ring opening. The bisulfitation reaction is carried out as an intermediate process step to adhesive synthesis as it renders the tannin molecules more soluble in water.

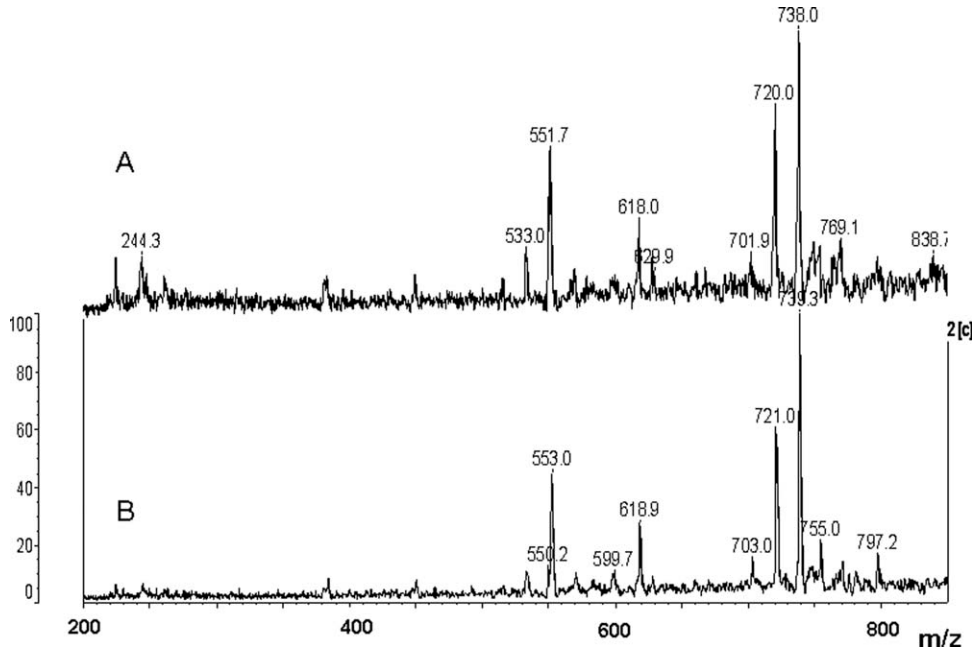

Figure 13. PSD fragmentation spectrum of the trimer with the mass of 905 Da from the bisulphited water-extracted mimosa tannin (sample 6), (A) with laser power of 130 and (B) laser power of 120.

The same type of CID analysis as performed for the cacao and quebracho tannins was also carried out for the mimosa tannin. The peak at m/z 905 was chosen as it was the most intense of the trimers. The mimosa tannin is the most complex of the tannins discussed in this work. Its angular nature and numerous possibilities of structures make the mass spectral assignment quite complicated. The fragmentation spectrum (Figure 11) looks uncomplicated; however, the mass differences observed here are not observed for the other oligomers.

In the case of the mimosa tannin, the mass of 152 Da is replaced by 168 Da, which clearly arises due to the presence of an additional hydroxyl group on the B ring. From m/z 552 to 244 the loss is 308 Da, and this clearly arises from a C-type unit. From theoretical calculation performed for the full assignment, the trimer at m/z 905 can be formed in two ways, first by two B-type units and a single C-type unit or two C-type units and an A-type unit. The loss of 168 Da from the precursor ion and also from the m/z 720 fragment ion shows the presence of a molecule with C-type units at each terminus. However, the fragment that appears at m/z 618 cannot be explained by the structure that is suggested here because it is separated by 287 Da from the precursor ion. This kind of behavior is explained by the quinone-methide mechanism, and from this, it seems that the molecule consists of a B-type terminus (see Figure 12). Following this explanation, the peak at m/z 618 can be assigned to an oligomer that consists of one B- and one C-type units. The mimosa tannin seems to follow the same fragmentation pattern as the cacao tannin, having both the RDA fission and quinone-methide mechanisms occurring simultaneously; however, in the case of the mimosa tannin, the mechanism is structure specific. From this information, it can be surmised that the trimer at m/z 905 in the MALDI-TOF spectrum is representative of two isomers, which seem to be present in the mimosa extract.

Similar to the quebracho sample, the mimosa tannin was also investigated using PSD (see Figure 13). In agreement with the previous results, similar fragmentation peaks were obtained.

CONCLUSIONS

MALDI-TOF-CID experiments have been demonstrated to be very useful for the monomer sequence determination and positive assignments of isobaric structures in wood-based complex tannin mixtures. Although standard MALDI-TOF MS measurements reveal the oligomer structure of these tannins, this method cannot distinguish between isomers, and therefore, ambiguous structural assignments are obtained in a number of cases. The actual microstructures present can unambiguously be identified by MALDI-TOF-CID.

REFERENCES

1. Pizzi, A. Wood Adhesives Chemistry and Technology; Marcel Dekker: New York, **1983.**
2. Pizzi, A. Advanced Wood Adhesives Technology; CRC Press: New York, **1994.**
3. White, T. *J. Sci. Food Agric.* **1957,** *8*, 377.
4. Hartzfeld, P. W.; Forkner, R.; Hunter, M. D.; Hagerman, A. E. *J. Agric. Food Chem.* **2002,** *50*, 1785.
5. Haslam, E. Practical Polyphenolics from Structure to Molecular Recognition and Physiological Action; Cambridge University Press: New York, **1998.**
6. Meikleham, N. E.; Pizzi, A. *J. Appl. Polym. Sci.* **1994,** *53*, 1547.
7. Zhang, L. L.; Lin, Y. M. *Molecules* **2008,** *13*, 2986.
8. Pasch, H.; Pizzi, A. *J. Appl. Polym. Sci.* **2002,** *85*, 429.
9. Pizzi, A.; Pasch, H.; Rode, K.; Giovando, S. *J. Appl. Polym. Sci.* **2009,** *113*, 3847.
10. Pasch, H.; Pizzi, A.; Rode, K. *Polymer* **2001,** *42*, 7531.
11. Navarette, P.; Pizzi, A.; Pasch, H.; Rode, K.; Delmotte, L. *Ind. Crops Prod.* **2010,** *32*, 105.
12. Oo, C. W.; Pizzi, A.; Pasch, H.; Kassim, M. J. *J. Appl. Polym. Sci.* **2008,** *109*, 963.
13. Perret, C.; Pezet, R.; Tabacchi, R. *Phytochem. Anal.* **2003,** *14*, 202.
14. Behrens, A.; Maie, N.; Knicker, H.; Koegel-Knabner, I. *Phytochemistry* **2003,** *62*, 1159.
15. Raust, J. A.; Bruell, A.; Moire, C.; Farcet, C.; Pasch, H. J. Chromatogr. *A* **2008,** *1203*, 207.
16. Montaudo, G.; Garozzo, D.; Montaudo, M. S.; Puglisi, C.; Samperi, F. *Macromolecules* **1995,** *28*, 7983.
17. Hanton, S. D. *Chem. Rev.* **2001,** *101*, 527.
18. Belgacem, O.; Bowdler, A.; Brookhouse, I.; Brancia, F. L.; Raptakis, E. *Rapid Commun. Mass Spectrom.* **2006,** *20*, 1653.
19. Jackson, A. I. T.; Jennings, K. R.; Scrivens, J. H. *J. Am. Soc. Mass Spectrom.* **1997,** *8*, 76.
20. Cotter, R. J.; Griffith, W.; Jelinek, C. J. Chromatogr. *B* **2007,** *855*, 2.
21. Cai, Y. Z.; Xing, J.; Sun, M.; Zhan, Z. Q.; Corke, H. *J. Agric. Food Chem.* **2005,** *53*, 9940.
22. Zhu, F.; Cai, Y. Z.; Xing, J.; Ke, J.; Zhan, Z.; Corke, H. *Rapid Commun. Mass Spectrom.* **2009,** *23*, 1678.
23. Li, Y.; Hoskins, J. N.; Sreerama, S. G.; Grayson, M. A.; Grayson, S. M. *J. Mass Spectrom.* **2010,** *45*, 587.
24. Rigaud, J.; Escribano-Bailon, M. T.; Prieur, J.; Souquet, J. M.; Cheynier, V. J. Chromatogr. *A* **1993,** *654*, 255.
25. Rohr, G. E.; Riggio, G.; Meier, B.; Sticher, O. *Phytochem. Anal.* **2000,** *11*, 113.
26. Zywicki, B.; Reemtsma, T.; Jekel, J. J. Chromatogr. *A* **2002,** *970*, 191.
27. Vivas, N.; Nonier, M. F.; Vivas de Gaulejac, N.; Absalon, C.; Bertrand, A.; Mirabel, M. Anal. Chim. *Acta* **2004,** *513*, 247.
28. Li, Y.; Hoskins, J. N.; Sreerama, S. G.; Grayson, S. M. *Macromolecules* **2010,** *43*, 6225.