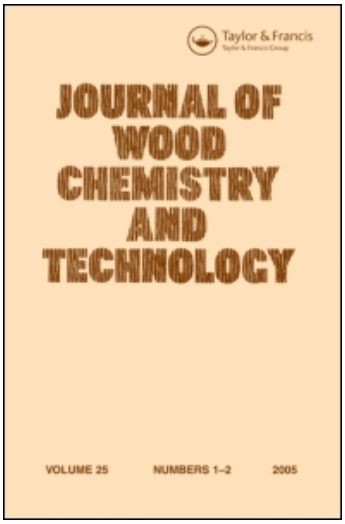


Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

Negative-Ion Fast-Atom-Bombardment Mass Spectrometry of Procyanidin Oligomers

J. J. Karchesy[a]; L. Y. Foo[b]; E. Barofsky[c]; B. Arbogast[c]; D. F. Barofsky[c]
[a] Department of Forest Products, Oregon State University, Corvallis, Oregon [b] Chemistry Division, D.S.I.R., Petone, New Zealand [c] Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon

To cite this Article Karchesy, J. J. , Foo, L. Y. , Barofsky, E. , Arbogast, B. and Barofsky, D. F.(1989) 'Negative-Ion Fast-Atom-Bombardment Mass Spectrometry of Procyanidin Oligomers', Journal of Wood Chemistry and Technology, 9: 3, 313 — 331
To link to this Article: DOI: 10.1080/02773818908050302
URL: http://dx.doi.org/10.1080/02773818908050302

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 9(3), 313-331 (1989)

NEGATIVE-ION FAST-ATOM-BOMBARDMENT MASS SPECTROMETRY OF PROCYANIDIN OLIGOMERS

J.J. Karchesy
Department of Forest Products
Oregon State University
Corvallis, Oregon 97331

L.Y. Foo
Chemistry Division, D.S.I.R.,
Private Bag, Petone, New Zealand

E. Barofsky, B. Arbogast and D.F. Barofsky
Department of Agricultural Chemistry
Oregon State University
Corvallis, Oregon 97331

ABSTRACT

The negative-ion fast-atom-bombardment (FAB) mass spectra of procyanidin oligomers are characterized by abundant $[M-H]^-$ ions and fragmentation that results primarily from retro-Diels-Alder fission and cleavage of the interflavanoid bond. B/E-linked scanning mass spectrometry established that sequence ions result from unimolecular gas-phase decompositions. A quinone-methide mechanism of interflavanoid bond cleavage is proposed to account for observed ions of different molecular weight originating from isomeric upper and lower flavan units. These ions can be used to establish the sequence of monomer units and also to distinguish a linear from a branched trimer.

INTRODUCTION

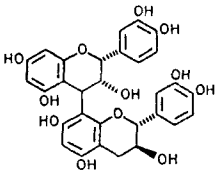
Fast atom bombardment (FAB) produces ions from large, labile, polar molecules as they are subjected to bombardment by energetic atoms.[1] The bombarding atoms (called primary

particles) usually have energies on the order of 10,000 eV. Among the ejected particles (called secondary particles) are ions of whole or intact analyte molecules (molecular ions) and ions of pieces of analyte molecules (fragment ions). When the analyte is an oligomer, fragment ions characteristic of the sequence of monomeric species in the molecule are referred to as sequence ions. A fragment ion resulting from decomposition of a secondary ion in flight in the mass analyzer is called a metastable ion.

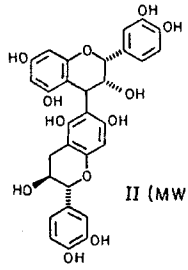
FAB produces both positive and negative ions that can be analyzed by mass spectrometry. Negative-ion FAB mass spectra sometimes resemble their positive-ion counterparts and provide useful complementary information about molecular weight and structure. For a given compound, one type of spectrum generally will be superior because of the number and magnitudes of structurally significant mass peaks. Since gas-phase decompositions of FAB-desorbed ions can mimic reactions in solution,[2,3] differences in positive- and negative-ion spectra can also reflect how a compound reacts under acid or base catalysis in solution chemistry.

We have previously reported on positive-ion FAB mass spectrometry and linked scanning mass spectrometry of procyanidin oligomers.[4] The positive-ion spectra are characterized by abundant molecular ions of the form $[M+H]^+$, fragment ions resulting from retro-Diels-Alder (RDA) fission of the monomeric flavanoid nucleus, and sequence ions resulting from cleavage of the interflavanoid (polymer) linkages. The sequence ions can be used to differentiate the two types of interflavanoid bonds found in procyanidins, as well as to distinguish a branched trimer from linear isomers. Metastable decomposition pathways established for the sequence ions show that cleavage of the B-type interflavanoid bonds--e.g., C4→C8, as in (I), or C4→C6, as in (II)--occurs in a one-step gas-phase unimolecular decomposition of the $[M+H]^+$ ion. The nature of

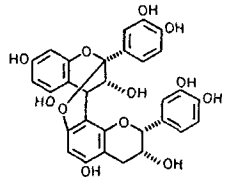
the sequence ions produced indicates that the gas-phase decomposition mimics the well-known, facile, acid-catalyzed cleavage of B-type linkages in solution chemistry. In contrast, sequence ions associated with A-type procyanidin bonds (e.g. (III)), which are resistant to acid-catalyzed cleavage in solution, are produced in a two-step process that requires an RDA fission of the $[M+H]^+$ ion before cleavage of the interflavanoid bond.



I (MW 578)



II (MW 578)



III (MW 576)

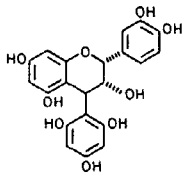
Negative-ion FAB-MS has been recently used to assist in both the structural elucidation and the analysis of mixtures of procyanidin oligomers, glucosylated flavanoid oligomers, and derivatives.[5,6,7,8] In particular, Self et al.[5] observed major fragments corresponding to RDA fission $[M-H-152]^-$ and to sequence ions produced by cleavage of the interflavanoid bond in C4→C8-linked procyanidins, in addition to abundant molecular-weight-defining $[M-H]^-$ ions.

In this study, we describe the use of negative-ion FAB mass spectrometry and linked scanning mass spectrometry to elucidate the structure of procyanidin oligomers and to establish the metastable decomposition pathways leading to the major fragment ions. Additionally, we demonstrate that the isomeric upper and lower flavan units produced from interflavanoid bond cleavage under negative-ion FAB mass spectrometry conditions can be distinguished and propose a mechanism to explain these results.

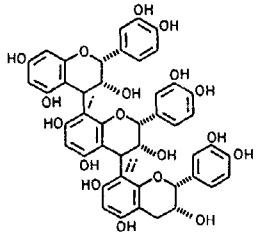
We also establish that the sequence ions produced can be used to distinguish a linear from a branched trimer.

RESULTS AND DISCUSSION

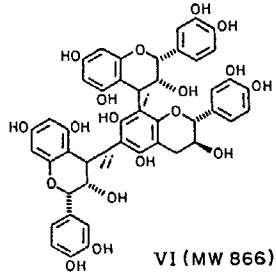
The negative-ion FAB mass spectra of procyanidins I-VI were characterized by abundant $[M-H]^-$ ions reflecting the relative stability of these phenols as anions (Table 1). Fragmentation occurred primarily by RDA fission and cleavage of the interflavanoid bond. Cleavage of the interflavanoid bond in dimers I-IV produced two monomeric sequence ions, one derived from the upper and the other from the lower flavan unit. In the linear trimer (V), two dimeric and two monomeric sequence ions were observed; the branched trimer (VI) produced only one dimeric and two monomeric sequence ions. Ions were observed also in the trimer spectra associated with RDA fission of the dimer sequence ions.



IV (MW 414)



V (MW 866)



VI (MW 866)

The type of data obtained for these compounds is exemplified by a typical negative-ion FAB mass spectrum of B-7 (II) (Fig. 1A). The spectrum of B-1 (I) is qualitatively very similar; slight differences in relative intensities of the principal peaks are probably caused by different locations of the interflavanoid bond. The $[M-H]^-$ ion at m/z 577 is readily apparent. RDA fission of the $[M-H]^-$ ion gives an $[M-H-152]^-$

TABLE 1

Masses and Percent Relative Abundance (In Parentheses; Normalized to the Most Abundant Sample Peak) of Major Ions in Negative-Ion FAB Mass Spectra of Procyanidin Oligomers.[a]

Compound	Ions observed				
	$[M-H]^-$	RDA	RDA-H_2O	Monomeric	Dimeric
I[b]	577(100)	425(25)	407(11)	287(12),289(18)	---
II[b]	577(100)	425(29)	407(8)	287(8),289(24)	---
III	575(100)	423(25)	---	285(19),289(10)	---
IV	413(100)	261(14)	---	287(7),125(14)	---
V	865(100)	713(14)	695(4)	287(17),289(20)	575(9),577(10)
		425(10)	407(10)		
		423(7)	405(4)		
VI	865(60)	713(13)	695(6)	287(20),289(15)	577(100)
		425(29)	407(19)		

[a] Magic bullet matrix.
[b] An additional ion peak coincided with the matrix ion peak at m/z 273 (-).

ion (m/z 425), which subsequently loses H_2O to give an ion at m/z 407. Cleavage of the interflavanoid bond leads to two monomeric sequence ions at m/z 289 and 287. The m/z 289 peak (Fig. 1A), which is rationalized as originating from the lower monomer unit (DEF), is about three times more intense than the m/z 287 peak, which originates from the upper monomer unit (ABC) in I and II. The peaks at m/z 217, 273 and 307 result from ions from the magic bullet matrix.

In order to establish the metastable decomposition pathways for the compounds studied here, B/E-linked scanning was carried out on their $[M-H]^-$, RDA, and sequence ions. Scans of the $[M-H]^-$ ion (m/z 577) and the $[M-H-152]^-$ RDA fragment ion

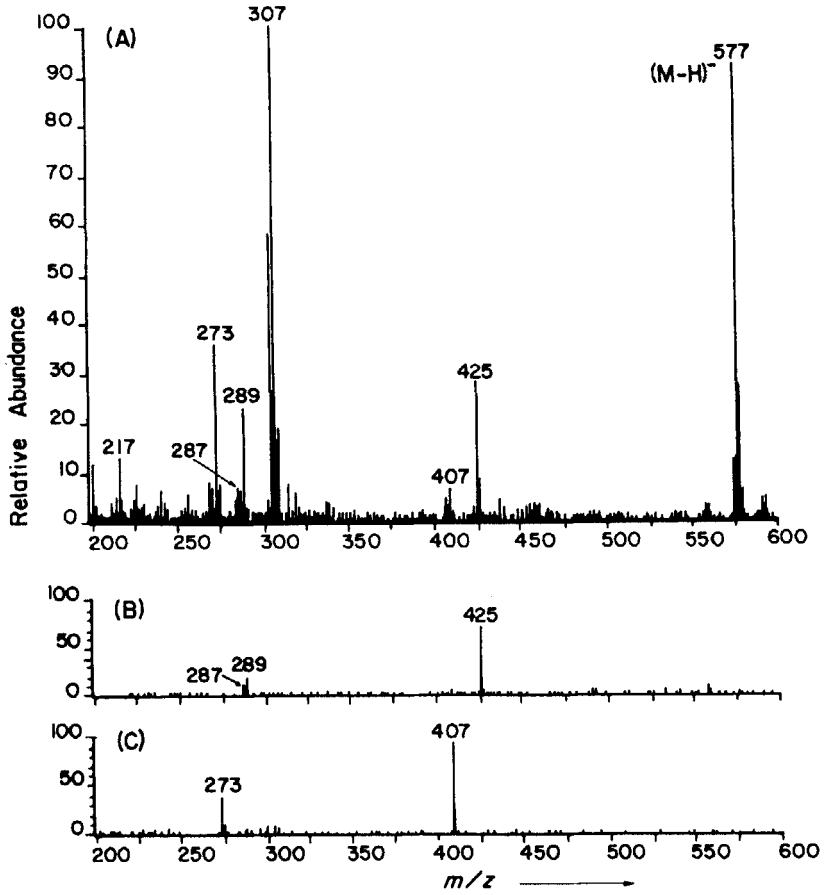
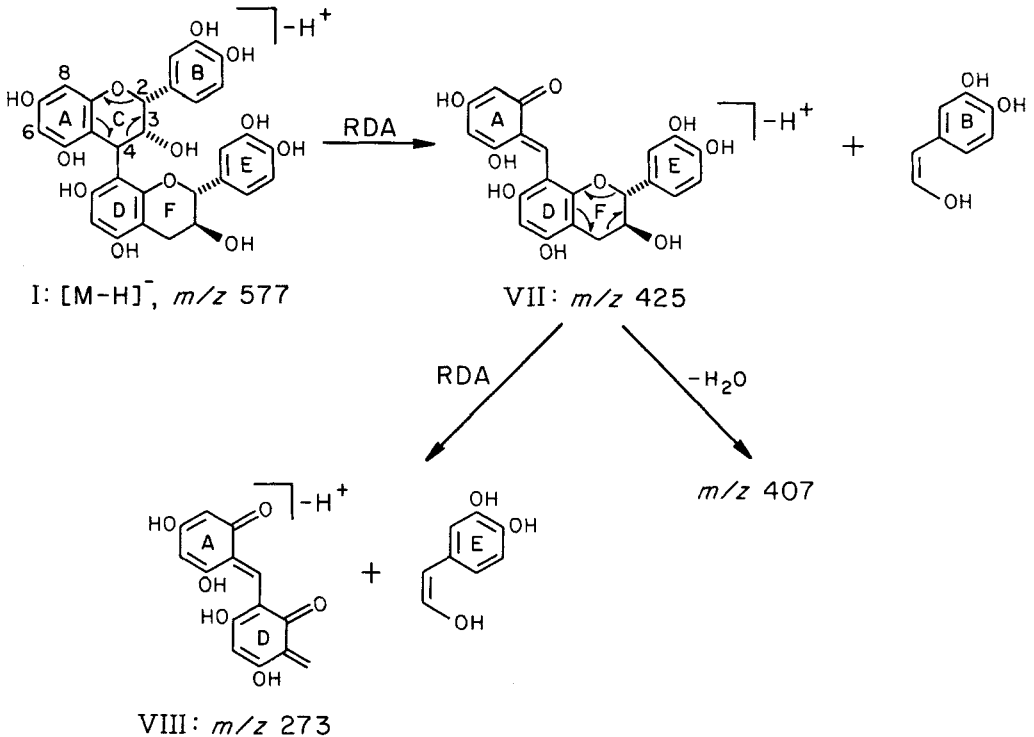


FIGURE 1. Typical negative-ion fast-atom-bombardment (FAB) mass spectra of B-7 (II): (A) regular spectrum, (B) daughter-ion (B/E) spectrum of m/z 577, $[M-H]^-$, (C) daughter-ion spectrum of m/z 425, $[M-H-152]^-$.

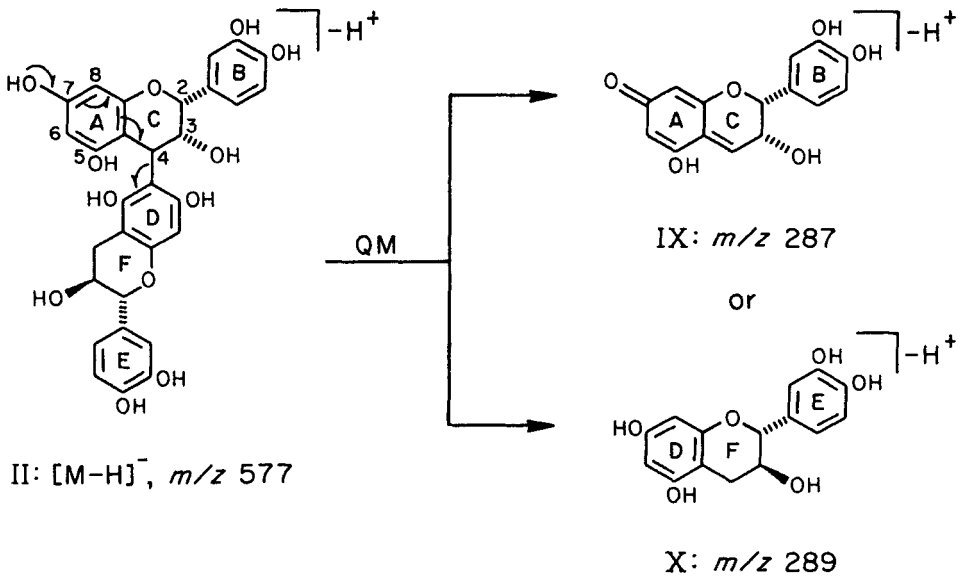


Scheme 1

Metastable decomposition pathway to retro-Diels-Alder (RDA) fragment daughter ions from the $[M-H]^-$ ion of I

(m/z 425) from B-7 (II) are shown in Figs. 1B and 1C respectively; very similar scans were obtained for the $[M-H]^-$ and RDA ions of B-1 (I). Again, slight differences in relative intensities of daughter ions likely resulted from different stereochemistries in the parent ion isomers. The $[M-H]^-$ ion (m/z 577) clearly undergoes unimolecular gas phase decompositions to give daughter ions at m/z 425 (RDA), 289 and 287 (Fig. 1B). This scan confirms that both the m/z 289 and 287 monomer ions come directly from the $[M-H]^-$ ion. As expected, the ion at m/z 425 loses H_2O to give a daughter ion at m/z 407 (Fig. 1C); unexpectedly, a second daughter ion, at m/z 273 is also produced from the m/z 425 ion (Scheme 1). This contribution to the m/z 273 peak was unnoticed in the regular spectra of both B-1 (I) and B-7 (II) because of the large m/z 273 peak from the magic bullet matrix. One might have observed the sample m/z 273 peak by using a different matrix. Thus, B/E-linked scans provide an additional advantage: portions of the spectrum can be observed without interference from matrix ions. The ion at m/z 273 is rationalized as a second consecutive RDA fission product, as shown in scheme 1 for B-1 (I); the same fragments occur for B-7 (II), except for the obvious difference attributable to the C4→C8 versus C4→C6 linkage.

A quinone-methide mechanism of interflavanoid bond cleavage is proposed to account for the two sequence ions produced from the $[M-H]^-$ ion of B-1 and B-7 (Scheme 2). Quinone-methide formation can occur either through the 7-O of (II) (Scheme 2) or through the 5-O (not shown). In order to confirm this mechanism, the possible metastable decomposition pathways for the $[M-H]^-$ ion of epicatechin-(4ß→2)-phloroglucinol (IV), which is unsymmetric about the interflavanoid bond, were closely examined (Scheme 3). Only the sequence ions at m/z 287 and 125 were observed, clearly indicating that the upper flavan unit

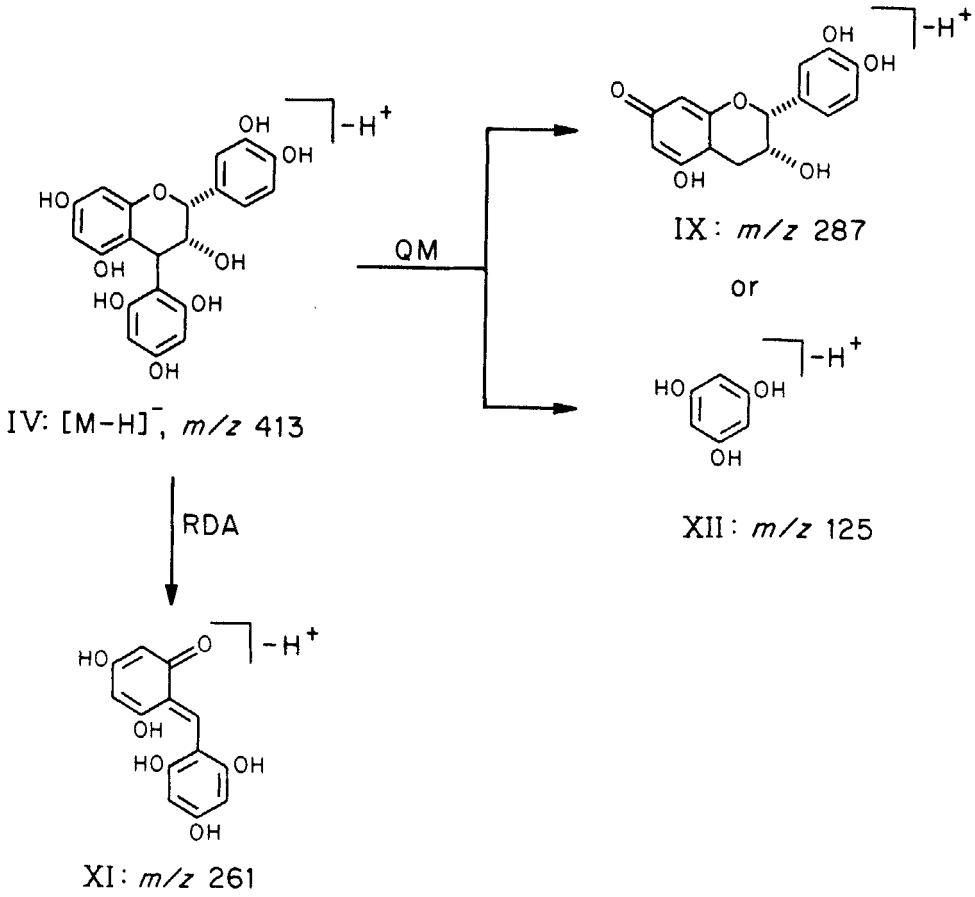


Scheme 2

Proposed quinone-methide (QM) mechanism for cleavage of B-type interflavanoid bond to produce sequence ions

exists after bond cleavage as the quinone-methide (IX) less a proton, while the lower unit exists as the phenol (XII) less a proton.

Scheme 2 is consistent with the sequence ions produced on cleavage of B-type interflavanoid linkages in both dimers and trimers (Table 1). Most importantly, this mechanism demonstrates that the upper and lower isomeric flavan units can be distinguished after cleavage of the bond between them. This has positive implications for future research on analytical methods that will identify the points of attachment for other



Scheme 3

Metastable decomposition pathways to major fragment ions in IV

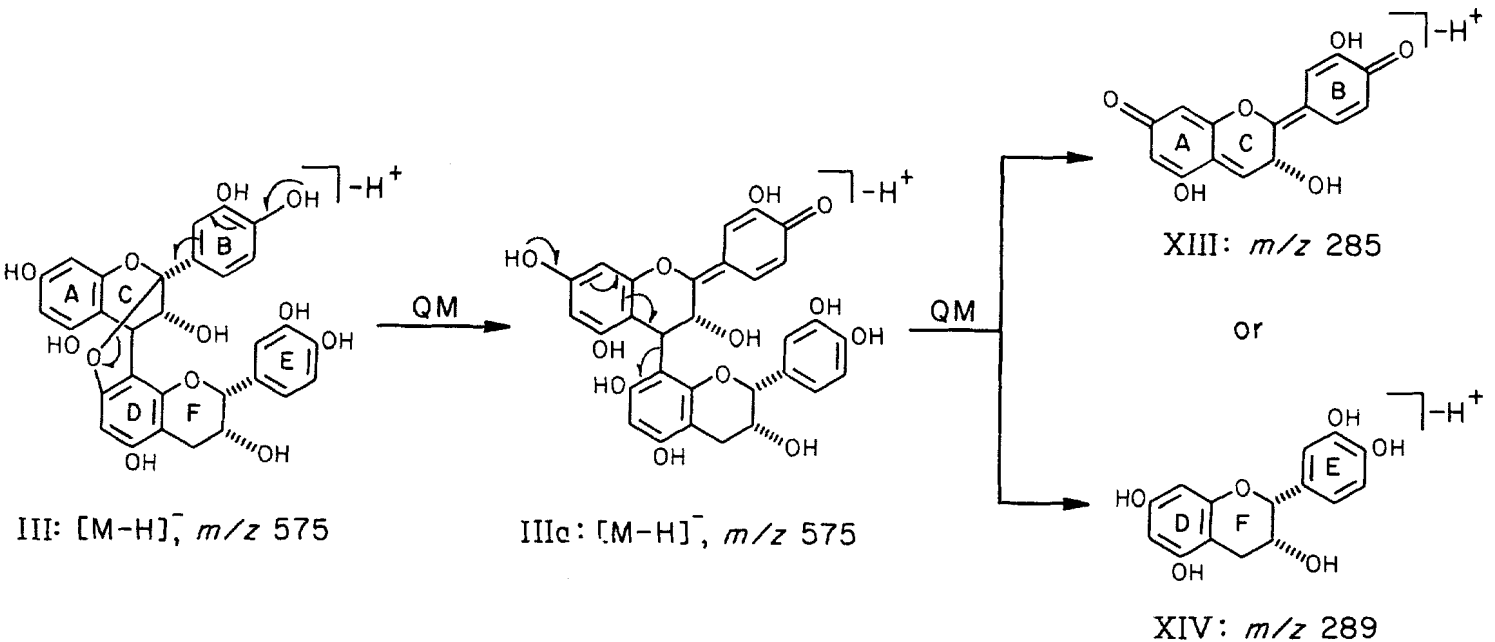
groups, such as glycosides or DNA adducts, on procyanidin oligomers.

Quinone-methide formation in solution chemistry has been proposed for compounds similar to those studied here. Attwood et al. demonstrated formation of quinone-methides for 5- and

7-hydroxyflavan-4-ols under both acid and alkaline conditions.[9] Foo and Wong proposed a quinone-methide intermediate in the solvolysis of diastereoisomeric leucoanthocyanidins under acidic conditions.[10] Laks and Hemingway have proposed a quinone-methide intermediate derived from the upper unit of a procyanidin polymer after solvolysis at alkaline pH.[11] Other research also indicates that procyanidins with good leaving groups at C-4 can undergo facile formation of quinone-methide intermediates under mild alkaline conditions.[12,13]

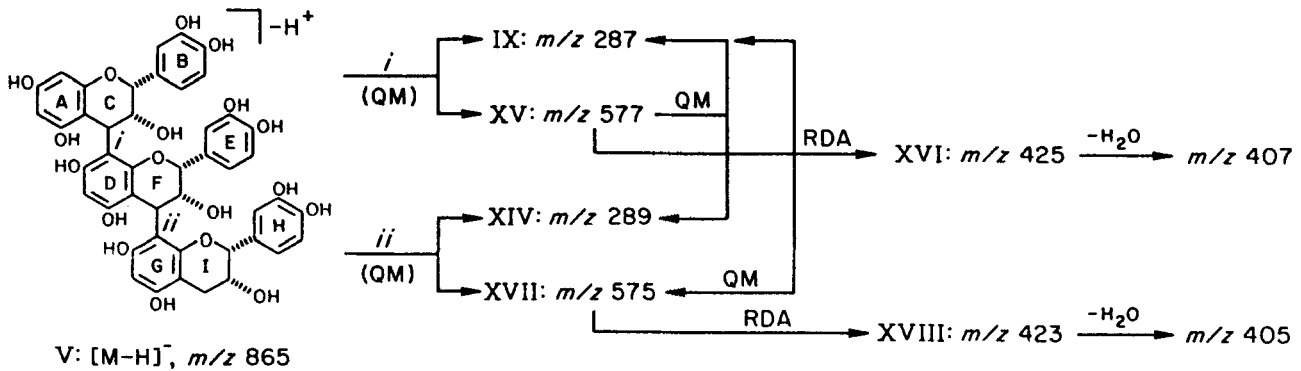
Cleavage of the A-type interflavanoid linkage in compound III is proposed to occur in two steps (Scheme 4). Quinone-methide formation through the B ring first opens the 7-O→2ß linkage to give IIIa, which is essentially a rearranged $[M-H]^-$ ion. IIIa then undergoes quinone-methide formation through the A ring to form either sequence ion XIII (m/z 285) or XIV (m/z 289). In support of this mechanism, Laks and Hemingway[11] have noted evidence for quinone-methide formation in the B ring of the flavan monomer catechin in solution. Also, Delcour and Tuytens[14] have proposed the inversion of prodelphinidin B-3 via a quinone-methide intermediate in the upper unit B ring, which leads to formation of prodelphinidin B-9.

Because the quinone-methide mechanism results in unsymmetric cleavage about the interflavanoid bond, one can also distinguish between a linear (V) and branched trimer (VI) by the dimer ions produced (Schemes 5 and 6). In the linear trimer (V), cleavage of the upper interflavanoid bond i gave the dimer ion XV (m/z 577), whereas cleavage of the lower interflavanoid bond ii gave the dimer ion XVII (m/z 575). Subsequent RDA fissions of each of these gave ions XVI (m/z 425) and XVIII (m/z 423) respectively. By contrast, cleavage of bond i in the branched trimer VI gave the dimer ion II (m/z 577) and cleavage



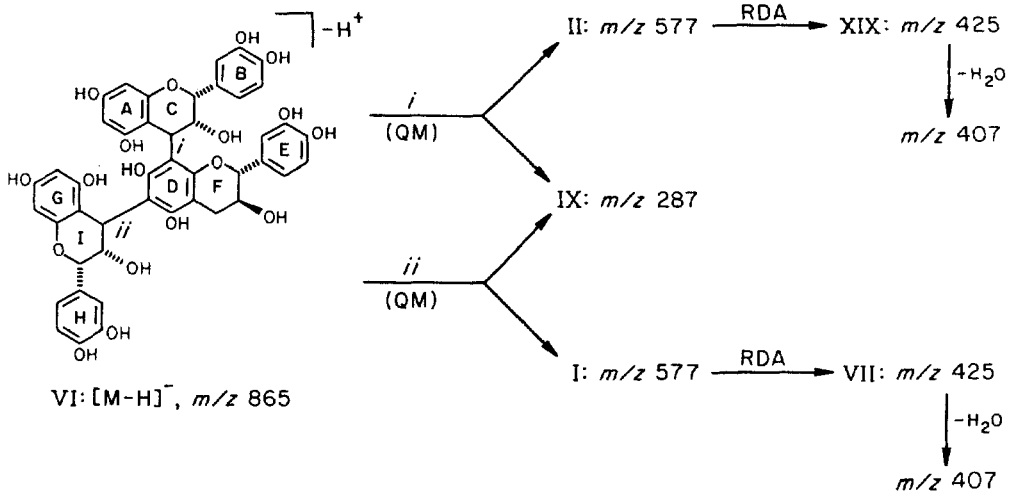
Scheme 4

Proposed quinone-methide (QM) mechanisms of interflavanoid cleavage in A-type linkages to give sequence ions



Scheme 5

Metastable decomposition pathways leading to major sequence ions for the linear trimer (V).



Scheme 6

Metastable decomposition pathways leading to major sequence ions for the branched trimer (VI): i, cleavage of the upper interflavanoid bond; ii, cleavage of the lower interflavanoid bond

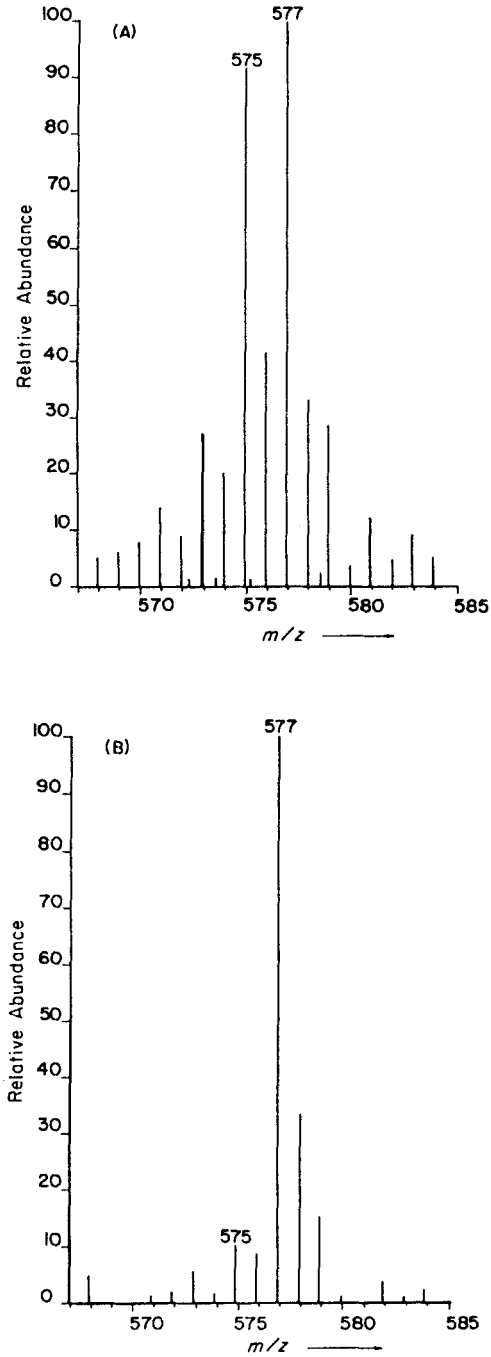
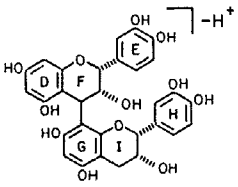
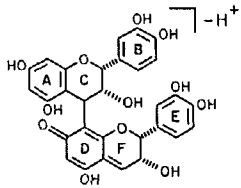


FIGURE 2. Dimer-regions of the mass spectra of (A) the linear trimer (V) and (B) the branched trimer (VI).

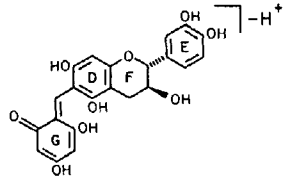
of bond ii gave the dimer ion I (m/z 577). Subsequent RDA fissions of I and II gave only an ion at m/z 425. Expanded views of the dimer ion region from typical negative-ion FAB mass spectra of V (Fig. 2A) and VI (Fig. 2B) are compared in Fig. 2; only the m/z 577 peak is significant for VI, while both m/z 575 and 577 peaks are significant for V.



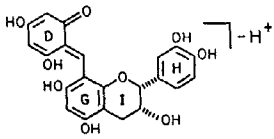
XV, *m/z* 577



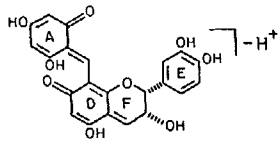
XVII, *m/z* 575



XIX, *m/z* 425



XVI, *m/z* 425



XVIII, *m/z* 423

CONCLUSIONS

Negative-ion FAB mass spectrometry of procyanidin oligomers complements positive ion FAB mass spectrometry and provides information about molecular weight, the type of interflavanoid bond, and the sequence of monomer units. Both RDA ions and sequence ions arise from unimolecular gas-phase decompositions of parent $[M-H]^-$ ions. The quinone-methide mechanism proposed to account for cleavage of the interflavanoid bond, leading to the observed sequence ions, may not be limited to gas-phase reactions of procyanidins. The sequence ions described here mimic the proposed intermediates for interflavanoid bond

cleavage of procyanidins in solution chemistry under alkaline catalysis.

EXPERIMENTAL

The dimeric procyanidins B-1, epicatechin-(4ß→8)-catechin (I), and B-7, epicatechin-(4ß→6)-catechin (II), and the branched trimer epicatechin-(4ß→8)-catechin-(6→4ß)-epicatechin (VI) were synthesized as described elsewhere.[13,15] The linear trimer epicatechin-(4ß→8)-epicatechin-(4ß→8)-epicatechin (V) was isolated from Douglas-fir bark.[16] Epicatechin-(4ß→2)-phloroglucinol (IV) was synthesized by reaction of Douglas-fir procyanidin polymer with phloroglucinol under acidic conditions.[17] Procyanidin dimer A-1, epicatechin-(4ß→8;2ß→O→7)-catechin (III), was isolated from Arachis hypogea.[8]

Mass spectra were obtained with either a VG 7070E-HF mass spectrometer or a Kratos MS-50TC mass spectrometer. Both instruments were equipped with conventional FAB sources with Ion Tech saddlefield atom guns. A 5:1 mixture of dithiothreitol and dithioerythritol (commonly referred to as magic bullet) was used as the FAB matrix liquid. Approximately 10 µg of sample compound was dissolved in about 1 µl of magic bullet for analysis; the samples were bombarded with 8 KeV xenon atoms. Metastable decomposition pathways were established by B/E-linked scanning without collisional activation.

ACKNOWLEDGMENTS

This research was supported by the USDA Competitive Research Grants Program for Forest and Rangeland Renewable

Resources (85-FSTY-0144), by the Forest Research Laboratory, Oregon State University, and by the Chemistry Division, DSIR, New Zealand. L.Y. Foo was a visiting scientist at Oregon State University when this research was carried out. This is paper number 2422 of the Forest Research Laboratory.

REFERENCES

1. C. Fenselau and R.J. Cotter, Chem. Rev., 87, 501 (1987).

2. F.W. McLafferty (ed.), Tandem Mass Spectrometry, Wiley-Interscience, New York, 1983.

3. A.L. Burlingame and N. Castagnoli, Jr. (eds), Mass Spectrometry in the Health and Life Sciences, Chapts. 9, 10, 12-14, Elsevier, New York, 1985.

4. J.J. Karchesy, R.W. Hemingway, L.Y. Foo, E. Barofsky and D.F. Barofsky, Anal. Chem., 58, 2563 (1986).

5. R. Self, J. Eagles, G.C. Galletti, I. Mueller-Harvey, R.D. Hartley, A.G.H. Lea, D. Magnolato, U. Richli, R. Giijer and E. Haslam, Biomedical and Environmental Mass Spectrometry, 13, 449 (1986).

6. R. Gujer, D. Magnolato and R. Self, Phytochemistry, 25, 1431 (1986).

7. G. Galletti and R. Self, Annoli di chimica, 76, 195 (1986).

8. J.J. Karchesy and R.W. Hemingway, J. Agric. Food Chem., 34, 966 (1986).

9. M.R. Attwood, B.R. Brown, S.G. Lisseter, C.L. Torrero and P.M. Weaver, J. Chem. Soc. Chem. Commun., 177 (1984).

10. L.Y. Foo and H. Wong, Phytochemistry, 25, 1961 (1986).

11. P.E. Laks and R.W. Hemingway, J. Chem. Soc. Perkin Trans. 1, 465 (1987).

12. R.W. Hemingway and L.Y. Foo, J. Chem. Soc. Chem. Commun., 1035 (1983).

13. L.Y. Foo and R.W. Hemingway, J. Chem. Soc. Chem. Commun., 85 (1984).

14. J.A. Delcour and G.M. Tuytens, J. Inst. Brewing, 90, 153 (1984).

15. R.W. Hemingway, J.J. Karchesy, G.W. McGraw and R.A. Wielsek, Phytochemistry, 22, 275 (1983).

16. L.Y. Foo and J.J. Karchesy, Phytochemistry, in press.

17. L.Y. Foo and L.J. Porter, J. Chem. Soc. Perkin 1, 1186 (1978).