

# FAST ATOM BOMBARDMENT MASS SPECTROMETRY OF CONDENSED TANNIN SULFONATE DERIVATIVES<sup>1</sup>

*J. J. Karchesy and L. Y. Foo*

Assistant Professor and Visiting Scientist  
Department of Forest Products, Oregon State University  
Corvallis, OR 97331

*R. W. Hemingway*

Project Leader  
Forest Products Utilization Research, Southern Forest Experiment Station  
Pineville, LA 71360

*E. Barofsky and D. F. Barofsky*

Research Assistant and Associate Professor  
Department of Agricultural Chemistry, Oregon State University  
Corvallis, OR 97331

(Received April 1988)

## ABSTRACT

Condensed tannin sulfonate derivatives were studied by fast atom bombardment mass spectrometry (FAB-MS) to assess the feasibility of using this technique for determining molecular weight and structural information about these compounds. Both positive- and negative-ion spectra provided useful data with regard to molecular weight, cation species present, and presence of the sulfonate moiety. Additional structural information was provided in the spectra of the dimer sulfonates by fragment ions resulting from retro-Diels Alder fission and cleavage of the interflavanoid bond. Overall, negative-ion spectra proved to be superior to positive-ion spectra because of less interference from matrix ions. FAB-MS holds promise as a technique for analyzing condensed tannin sulfonates yet to be isolated and will help facilitate development of new adhesives made with these compounds.

*Keywords:* Condensed tannins, sulfonate derivatives, fast atom bombardment, mass spectrometry, bark chemicals.

## INTRODUCTION

Condensed tannin sulfonate derivatives have considerable potential for use in the development of new wood adhesives. Good yields of tannin sulfonates can be obtained relatively inexpensively from conifer bark (Hemingway and Lloyd 1982). They are extremely reactive to condensation with formaldehyde and therefore might replace not only phenol in phenol-formaldehyde resins but also resorcinol in phenol-resorcinol-formaldehyde resins (Kreibich and Hemingway 1987). In addition, tannin sulfonates have some significant advantages over tannins extracted with neutral solvents; they are more water-soluble and less viscous and, on curing under basic conditions, easily lose the sulfonate group to become water-insoluble.

Foo et al. (1983) have shown that sulfonation of southern pine bark tannins

---

<sup>1</sup> This is Paper 2344 of the Forest Research Laboratory, Oregon State University, Corvallis, Oregon. Mention of tradenames or commercial products does not constitute endorsement by the authors or their institutions.

involves cleavage of the polymer linkages, generating epicatechin-4-sulfonate (I), a minor amount of catechin-2-sulfonate (II), and low molecular weight oligomers such as the dimer sulfonates (III) and (IV). However, most condensed tannin sulfonates remain uncharacterized. Procedures normally used for the analysis of tannins, such as molecular weight determination by gel permeation chromatography of the acetates (Williams et al. 1983) or separation by reversed-phase high-pressure liquid chromatography, have not worked well with the sulfonate derivatives, probably because the sulfonate functional group is highly water-soluble.

In the study reported here, we examined the condensed tannin sulfonate derivatives I-IV and the structurally related compounds V and VI by fast atom bombardment mass spectrometry (FAB-MS) (Barber et al. 1982), a relatively new technique for mass analyzing nonvolatile, thermally labile materials not amenable to traditional mass spectrometric methods that require samples to be volatilized before they can be ionized. In a previous study, we have reported the use of FAB-MS as a tool for sequencing procyanidin oligomers, for differentiating between the various types of polymer linkages present, and for distinguishing between branched and linear trimers (Karchesy et al. 1986). For more detailed description of FAB-MS and its applications, the interested reader is referred to the recent review by Burlingame et al. (1986).

#### MATERIALS AND METHODS

Compounds I-IV were obtained from the sulfonation of pine bark tannins as previously described (Foo et al. 1983), compounds V and VI from the sulfonation of 2-hydroxybenzyl alcohol and the sulfomethylation of phloroglucinol (McGraw et al. 1988).

Samples were prepared by adding the compound, either as a solid or in a methanol solution, to a drop of glycerol on the target probe. Positive-ion spectra for compounds I-VI in a glycerol matrix were obtained with a Varian CH-7 mass spectrometer modified to accept an Ion Tech Ltd. saddle field atom gun. Negative-ion spectra for compounds I, II, IV, and V were obtained with a Hitachi RMU-6E, also modified to operate in the FAB mode but with the added capability of negative-ion detection. In both cases, samples were bombarded with 7 keV xenon atoms. Spectra were calibrated with CsI.

High-resolution positive- and negative-ion spectra for compound III were obtained with both a VG 7070E-HF mass spectrometer and a KRATOS MS-50 TC mass spectrometer. For these spectra, samples were prepared by dissolving compound III into a 5:1 mixture of dithiothreitol and dithioerythritol (Magic Bullet) directly on the target of the sample insertion probe. Samples were bombarded with 8 keV xenon atoms. Only slight differences in the relative heights of various ion peaks were observed between spectra produced from the glycerol and Magic Bullet matrices (Table 1), and these differences did not affect qualitative interpretation of the spectra.

Gas-phase metastable decomposition pathways to daughter ions were established by B/E linked scanning without collisional activation.

#### RESULTS AND DISCUSSION

The positive-ion FAB mass spectra of compounds I-VI are characterized by ion peaks corresponding to  $[M+Na]^+$ ,  $[M+H]^+$ ,  $[M-HSO_3]^+$ , and  $[M-NaSO_3]^+$

TABLE 1. Principal ions observed in positive and negative FAB mass spectra.

Com- pound	Matrix	Ions observed <sup>a</sup>				
		Positive-ion spectra				
		[M+Na] <sup>+</sup>	[M+H] <sup>+</sup>	[M-HSO <sub>3</sub> ] <sup>+</sup>	[M-NaSO <sub>3</sub> ] <sup>+</sup>	Other
I	Glycerol	415 (100)	393 (6)	311 (47)	289 (7)	271 (10)
II	Glycerol	417 (100)	395 (56)	313 (26)	291 (16)	273 (g)
III	Magic Bullet	703 (16)	681 (12)	599 (37)	577 (—) <sup>b</sup>	415 (24), 393 (21), 311 (100), 293 (19), 289 (26), 271 (19)
IV	Glycerol	703 (60)	681 (12)	599 (23)	577 (—) <sup>b</sup>	415 (24), 393 (21), 311 (100), 293 (36), 289 (11), 271 (27)
V	Glycerol	233 (100)	211 (9)	129 (14)	107 (19)	
VI	Glycerol	265 (30)	243 (30)	161 (23)	139 (100)	221 (38)
		Negative-ion spectra				
		[M-H] <sup>-</sup>	[M-Na] <sup>+</sup>	[M-Na-O] <sup>-</sup>	Other	
I	Glycerol	391 (25)	369 (100)	353 (9)		
II	Glycerol	393 (25)	371 (100)	355 (8)	289 (14)	
III	Magic Bullet	679 (70)	657 (100)	641 (10)	575 (7), 527 (21), 505 (10), 369 (21)	
IV	Glycerol	679 (75)	657 (100)	641 (13)	575 (9), 527 (7), 505 (12), 369 (18)	
V	Glycerol	209 (26)	187 (100)	171 (14)		

<sup>a</sup> Nominal  $m/z$  (relative abundance in percent of most abundant sample ion).

<sup>b</sup> Less than 5% relative abundance.

(Table 1, Fig. 1). This series of ion peaks can be used to establish molecular weight, cation species present, and presence of the sulfonate moiety. In contrast to the compounds studied here, which are all benzylic sulfonates, the FAB mass spectra of certain aromatic sulfonates show weak ion peaks corresponding to loss of sulfite ( $\text{SO}_3^-$ ) as  $[\text{M}+\text{Na}-\text{SO}_3]^+$  and  $[\text{M}+\text{H}-\text{SO}_3]^+$  (Monaghan et al. 1982a, b). The relatively high abundance of  $[\text{M}-\text{NaSO}_3]^+$  for compound VI is probably due to the highly resonance-stabilized carbonium ion VII; compound VI also produces a relatively high abundance peak at  $m/z$  221, indicating displacement of the sodium atom by a hydrogen atom, i.e.,  $[\text{M}-\text{Na}+2\text{H}]^+$ . For compounds I and II,  $[\text{M}-\text{NaSO}_3]^+$  readily loses water to give ion peaks at  $m/z$  271 (VIII) and  $m/z$  273 (IX), respectively; these are also highly resonance-stabilized carbonium ions.

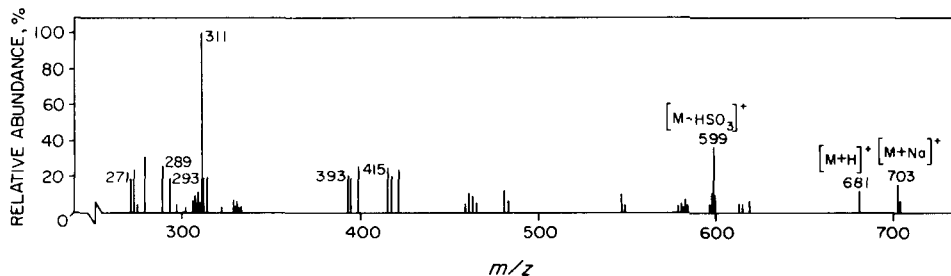


FIG. 1. Positive-ion FAB mass spectrum (background subtracted) of sodium epicatechin (4 $\beta$ -8)-epicatechin (4 $\beta$ ) sulfonate (III) in Magic Bullet. Abundances are normalized to most abundant sample ion; unlabeled peaks cannot be accounted for.

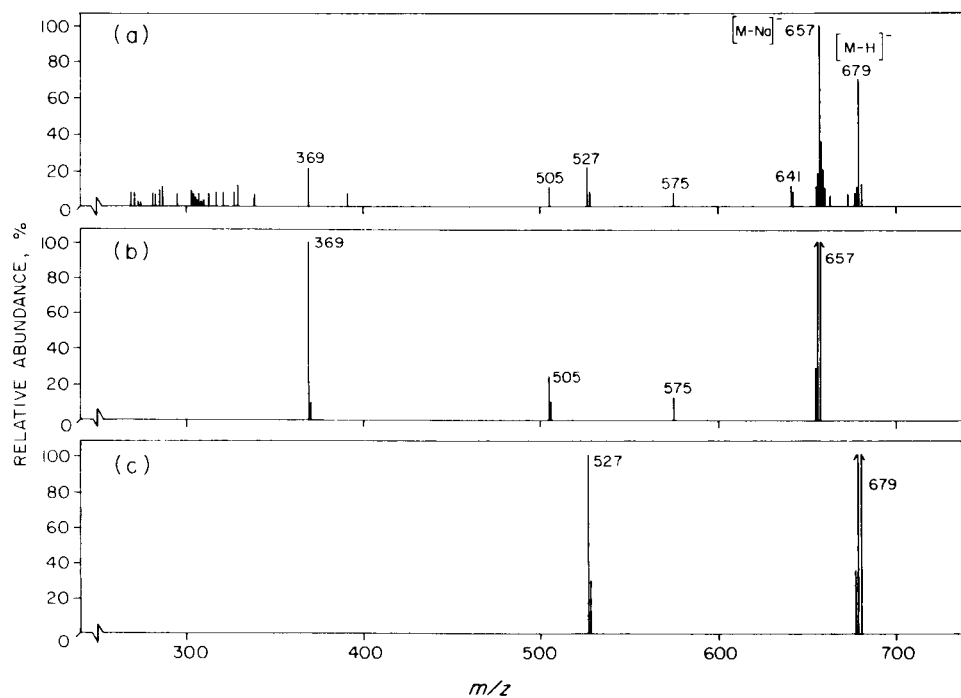
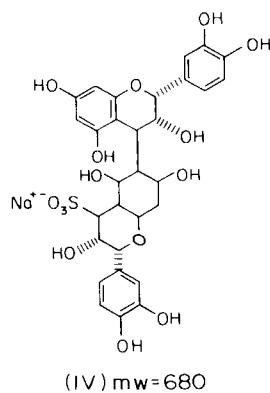
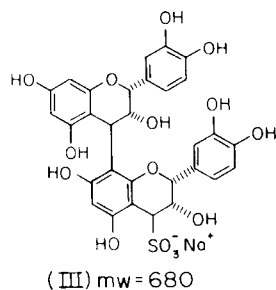
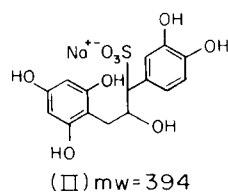
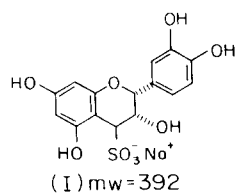
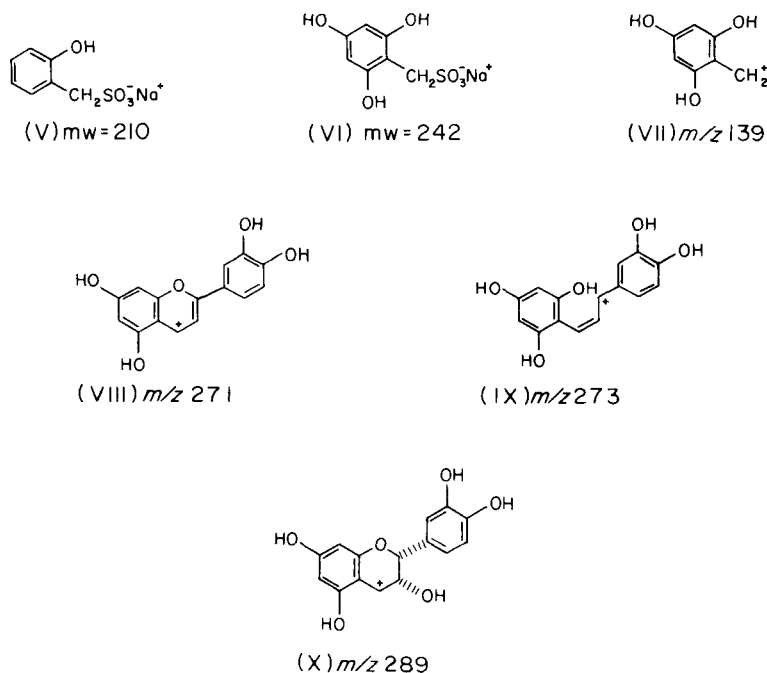


FIG. 2. (a) Negative-ion FAB mass spectrum (background subtracted) of sodium epicatechin (4 $\beta$ -8)-epicatechin (4 $\beta$ ) sulfonate (III) in Magic Bullet. Abundances are normalized to the most abundant sample ion; unlabeled peaks cannot be accounted for. Linked-scan spectra showing daughter ions resulting from metastable decomposition of (b) the [M-Na]<sup>-</sup> ion ( $m/z$  657) and (c) the [M-H]<sup>-</sup> ion ( $m/z$  679). Abundances are normalized to the most abundant daughter ion.

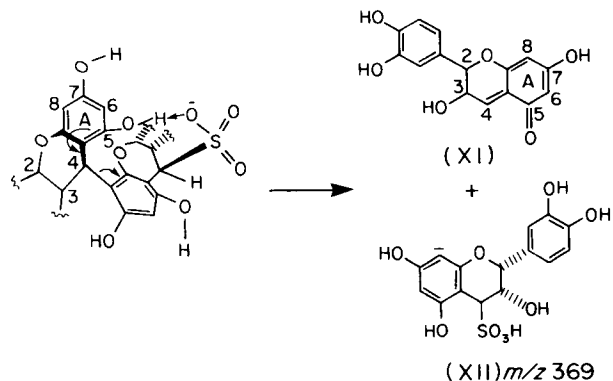




The dimer sulfonates III and IV exhibit substantial fragmentation in their positive-ion spectra (Table 1, Fig. 1). The ease with which the interflavanoid bond cleaves by either acid or base catalysis in solution chemistry is well known (Haslam 1982; Laks and Hemingway 1987); under FAB-MS conditions, this cleavage leads to ion peaks based on the individual flavan units from which the oligomers are built (Karchesy et al. 1986). Thus, in the FAB-MS spectra of the dimers III and IV, ion peaks are observed at  $m/z$  415, 393, 311, and 289, corresponding to the terminal unit, epicatechin-4-sulfonate (I), as  $[M_1 + Na]^+$ ,  $[M_1 + H]^+$ ,  $[M_1 - HSO_3]^+$ , and  $[M_1 - NaSO_3]^+$ , respectively (Table 1, Fig. 1). The ion peak at  $m/z$  289 can also arise from the upper monomer unit as the carbonium ion (X) by direct acid-catalyzed cleavage of the interflavanoid bond of either III or IV (Foo et al. 1983; Karchesy et al. 1986).

Linked-scan spectra (daughter ion) obtained from the  $m/z$  703, 681, and 599 ions of the dimers III and IV show no significant peaks at  $m/z$  415, 393, and 311, respectively. This observation indicates that the reactions leading to the latter ion species, as well as the  $m/z$  289 ion, are occurring in the liquid matrix on the FAB probe rather than in the gas phase as unimolecular decompositions.

The negative-ion spectra of the condensed tannin sulfonates exhibit less interference from matrix ions than do the positive-ion spectra. This difference is readily apparent when Fig. 2a, a negative-ion spectrum for the dimer sulfonate III, is compared with Fig. 1. The increased signal-to-noise ratio in the negative-ion spectra is due to greater stability of the sulfonate and phenolic anions relative to the FAB matrix ions. Thus, strong molecular ion peaks corresponding to  $[M - Na]^-$  and  $[M - H]^-$  are observed in the spectra of each sulfonate studied (Table 1, Fig. 2a). As in the case of positive ionization, the monomers produce little or no



Scheme 1

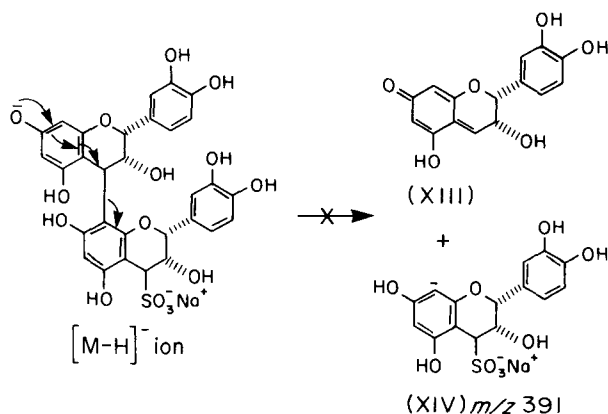
fragmentation, whereas the dimers III and IV yield ions that can be used to gain structural information.

Two major fragment ions are observed in Fig. 2a. The ion peak at  $m/z$  527,  $[M-H-152]^-$ , corresponds to a retro-Diels Alder fission of the flavanoid nucleus of the  $[M-H]^-$  ion ( $m/z$  679); retro-Diels Alder fission is commonly observed in FAB (de Koster et al. 1985; Karchesy et al. 1986) and other types of mass spectra of flavanoid compounds (Mabry and Markham 1975). The ion peak at  $m/z$  369 apparently originates from the lower flavanoid unit by cleavage of the interflavanoid bond in III (Fig. 2a).

The mechanism of interflavanoid bond cleavage and formation of the  $m/z$  369 ion is rationalized on the basis of the intramolecular reaction shown in Scheme 1. The sulfonate oxygen anion of  $[M-Na]^-$  abstracts the proton from the 5-hydroxyl of the upper flavanoid unit, forming the quinone methide (XI) from the upper unit; cleavage of the interflavanoid bond forms the anion XII from the lower unit. This mechanism is consistent both with the known liability of the procyanidin interflavanoid bond under basic conditions in solution chemistry (Laks and Hemingway 1987) and with molecular geometry (Drieding models show that rotation about the interflavanoid bond brings the sulfonate oxygens to within 1.5 angstroms of the 5-hydroxyl proton of the upper unit, sufficiently close for the reaction to occur).

In contrast to the case for  $[M-Na]^-$ , base-catalyzed cleavage of the interflavanoid bond of III in its  $[M-H]^-$  form ( $m/z$  679) does not readily occur under FAB-MS conditions, as evidenced by the lack of an ion peak at  $m/z$  391 (Scheme 2). Although formation of the quinone methide (XIII) is expected to be favorable, formation of the anion XIV is deemed unfavorable because of the proximity of two negative charges, one on the phenolic A ring and the other on the sulfonate salt moiety that presumably remains ionic in the gas phase.

Linked-scan (B/E) spectra of  $[M-H]^-$  ( $m/z$  679) and  $[M-Na]^-$  ( $m/z$  657) produced from III established the metastable decomposition pathways to their daughter ions. These daughter-ion spectra (Fig. 2b, c) also confirm that the  $m/z$  527 ion arises from the unimolecular gas-phase decomposition of  $[M-H]^-$ , the  $m/z$  369 ion from the unimolecular gas-phase decomposition of  $[M-Na]^-$ . Additionally, these spectra show that  $[M-Na]^-$  is the parent to daughter ions at  $m/z$  575 and 505. The ion at  $m/z$  575 corresponds to loss of  $H_2SO_3$ , that at  $m/z$  505 to a



Scheme 2

retro-Diels Alder fission,  $[M-Na-152]^-$ . The ion peaks at  $m/z$  575 and 505 are not major ones in the regular FAB-MS of III; linked scanning permits them to be observed by eliminating the background ion peaks due to the Magic Bullet matrix. Significant matrix ion peaks are observed for Magic Bullet under negative FAB-MS conditions at  $m/z$  217, 273, and 307.

#### CONCLUSIONS

The molecular weights of condensed tannin sulfonate derivatives can be readily obtained by positive- or negative-ion FAB-MS to give  $[M+H]^+$  and  $[M+Na]^+$  or  $[M-H]^-$  and  $[M-Na]^-$ , respectively. The negative-ion spectra were found superior to the positive-ion spectra because they had less interference from matrix ions. In the case of the dimer sulfonates, structural information was obtained from ion peaks resulting from cleavage of the interflavanoid bond. FAB-MS holds promise for applications in the analysis of condensed tannin sulfonate derivatives yet to be isolated and the adhesive resins made with them.

#### REFERENCES

- BARBER, M., R. S. BORDOLI, G. J. ELLIOTT, R. D. SEDGWICK, AND A. N. TAYLOR. 1982. Fast atom bombardment mass spectrometry. *Anal. Chem.* 54:645-657.
- BURLINGAME, A. L., T. A. BAILLIE, AND P. J. DERRICK. 1986. Mass spectrometry. *Anal. Chem.* 58:165R-211R.
- DEKOSTER, C. G., W. HEERMA, G. DIJKSTRA, AND G. J. NIEMANN. 1985. Fast atom bombardment of flavonols. *Biomed. Mass Spectrom.* 12:596-601.
- FOO, L. Y., G. W. MCGRAW, AND R. W. HEMINGWAY. 1983. Condensed tannins: Preferential substitution at the interflavanoid bond by sulfite ion. *J. Chem. Soc., Chem. Commun.* 12:672-673.
- HASLAM, E. 1982. Proanthocyanidins. Pages 417-447 in J. B. Harborne and T. J. Mabry, eds. *The flavanoids: Advances in research*. Chapman and Hall, London.
- HEMINGWAY, R. W., AND R. C. LLOYD. 1982. Economic prospects for conifer bark extracts. Final rept. 50-3201-47. USDA For. Serv., South. For. Exp. Stn., Pineville, LA.
- KARCHESY, J. J., R. W. HEMINGWAY, Y. L. FOO, E. BAROFKY, AND D. F. BAROFKY. 1986. Sequencing procyanidin oligomers by fast atom bombardment mass spectrometry. *Anal. Chem.* 58:2563-2567.
- KREIBICH, R. E., AND R. W. HEMINGWAY. 1987. Condensed tannin-sulfonate derivatives in cold-setting wood-laminating adhesives. *Forest Prod. J.* 37(2):43-46.
- LAKS, P. E., AND R. W. HEMINGWAY. 1987. Condensed tannins: Base-catalyzed reactions of polymeric

- procyanidins with toluene- $\alpha$ -thiol. Lability of the interflavanoid bond and pyran ring. *J. Chem. Soc. Perkin Trans. 1*:465-470.
- MABRY, T. J., AND K. R. MARKHAM. 1975. *Mass spectrometry of flavonoids*. Pages 78-126 in J. B. Harborne, T. J. Mabry, and H. Mabry, eds. *The flavonoids, part 1*. Academic Press, NY.
- MCGRAW, G. W., P. E. LAKS, AND R. W. HEMINGWAY. 1988. Condensed tannins: Desulfonation of hydroxybenzyl sulfonic acids related to proanthocyanidin derivatives. *J. Wood Chem. Tech.* 8(1): 91-109.
- MONAGHAN, J. J., M. BARBER, R. S. BARDOLI, R. D. SEDGWICK, AND A. N. TYLER. 1982a. Fast atom bombardment mass spectra of involatile sulphonated azo dyestuffs. *Org. Mass Spectrom.* 17:569-574.
- , ———, ———, ———, AND ———. 1982b. Fast atom bombardment mass spectra of involatile naphthalene sulphonic acids and sulphonate salts. *Org. Mass Spectrom.* 17:529-533.
- WILLIAMS, V. M., L. J. PORTER, AND R. W. HEMINGWAY. 1983. Molecular weight profiles of proanthocyanidin polymers. *Phytochemistry* 22(2):569-572.