

A New Method for Structural Study of Hydrolyzable Tannins by Negative Ion Fast Atom Bombardment Mass Spectrometry¹⁾

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Negative ion fast atom bombardment mass spectrometry employing a hexamethylphosphoric triamide–glycerol (1 : 1, v/v) mixture as a matrix has been found to facilitate the molecular weight determination of hydrolyzable tannins in their free forms, as well as the detection of diagnostic fragment ions.

Keywords fast atom bombardment mass spectrometry; hexamethylphosphoric triamide; electron pair donor; matrix; hydrolyzable tannin; ellagitannin; gallotannin

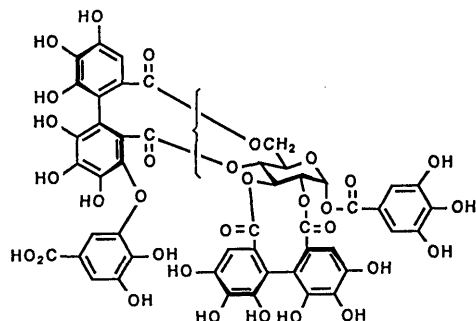
Introduction

As a promising aid for obtaining structural information from minute amounts of natural products, the role and effect of a matrix system on the ionization in fast atom bombardment mass spectrometry (FABMS) have been investigated in our laboratory.²⁾ Although various liquid matrix compounds have so far been reported,³⁾ analysis of non-volatile, highly polar and particularly acidic organic compounds has been successfully achieved by negative ion FABMS using a hexamethylphosphoric triamide (HMPA)–glycerol matrix system.⁴⁾ HMPA is well known as an ionizing solvent possessing high potential as an electron pair donor (EPD).⁵⁾ An EPD may act concurrently as a proton acceptor in the proton transfer reaction, whereas non-aqueous solvents such as HMPA never act as a proton donor in the same reaction. On the assumption that the

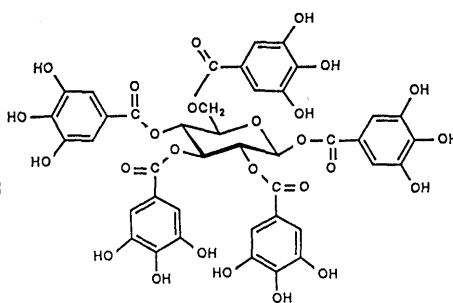
FAB ionization of highly polar organic molecules would occur through the interaction between the sample molecule and the matrix in the proton transfer reaction,⁶⁾ HMPA seems to be a useful matrix material for the generation of the deprotonated molecular ion, $[M-H]^-$. In addition, since in neutral solvents such as glycerol, the protons of phenolic hydroxyl groups in polyphenols are considered to tend to dissociate, we have chosen the HMPA–glycerol mixture as the matrix for analysis of tannins.

Results and Discussion

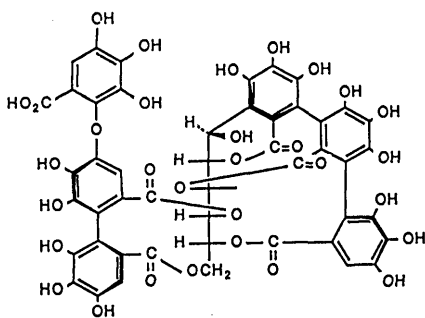
First, in order to compare the positive ion FABMS with the negative ion mode, we employed the ellagitannins, sanguins H-2 (**1**) and H-3 (**2**),^{7a)} as appropriate model compounds. The positive ion FAB mass spectrum of **1** revealed the $[M+H]^+$ ion peak at m/z 1105 with extremely



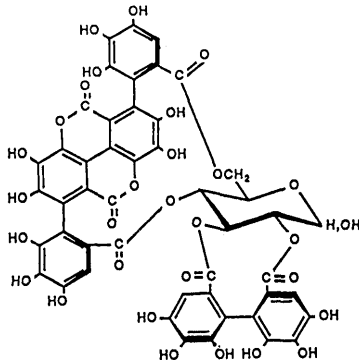
1 : MW 1104



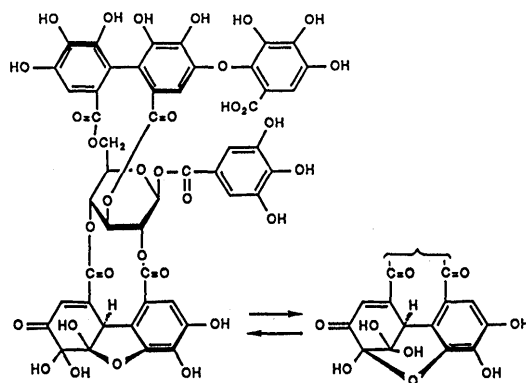
3 : MW 940



5 : MW 1102



4 : MW 1084



6 : MW 1120

low intensity, while the observation of the $[M+H]^+$ ion of **2** was difficult. On the other hand, the negative ion FAB mass spectra of **1** and **2** displayed prominent $[M-H]^-$ ion peaks in both cases. Figure 1 shows the negative ion FAB mass spectra of **2**, measured with glycerol and HMPA-glycerol matrix. Relatively high $[M-H]^-$ ion peaks were observed at m/z 1567 in both spectra, but the use of an HMPA-glycerol matrix resulted in a twentyfold increase in the absolute intensity of the $[M-H]^-$ ion compared with that in the case of glycerol alone. Furthermore, highly informative fragment ions appeared clearly in the lower HMPA-glycerol spectrum. The ions at m/z 1415, 1103, and 935 are due to the cleavages of ester and ether bonds as shown in Chart 1. The doubly charged molecular ion, $[M-2H]^{2-}$, is found at m/z 783. The ion at m/z 1265 is probably due to the elimination of a hexahydroxydiphenoyl (HHDP) group and the ion at m/z 633 is due to the fission of the ester bonds at C(4) and C(6) in the glucopyranose moiety. The most significant signal was observed at m/z 301 which may be attributable to the deprotonated ion of ellagic acid (Chart 2). Since it is known that ellagic acid is readily formed by hydrolysis of the HHDP group in ellagitannins, and that the signal can not be observed in the spectra of gallotannins, this signal is considered to be diagnostic for ellagitannins. Similarly, the signal at m/z 469 could be assigned to the deprotonated ion of sanguisorbic acid.

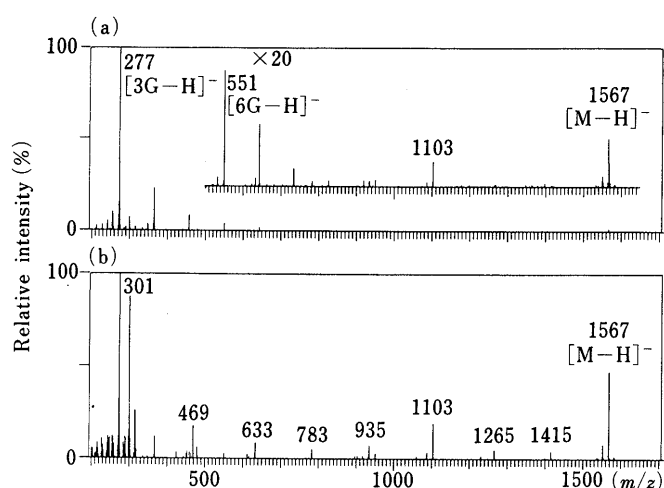


Fig. 1. The Negative Ion FAB Mass Spectra of Sanguini H-3 with (a) Glycerol and (b) HMPA-Glycerol Matrix
G, glycerol.

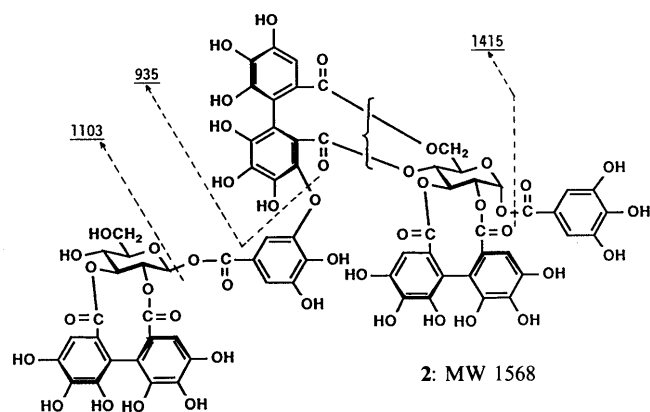


Chart 1

acid dilactone. Thus, it has become clear that by using an HMPA-glycerol mixture as a matrix, useful information about the structures of hydrolyzable tannins is obtainable without chemical hydrolysis.

The negative ion FAB mass spectra of several hydrolyzable tannins, *i.e.*, 1,2,3,4,6-pentagalloyl glucose (**3**),^{7b)} punicalagin (**4**),^{7c)} castavalonic acid (**5**),^{7d)} and mallotusinic acid (**6**),^{7e)} are displayed in Fig. 2. All the spectra clearly show the $[M-H]^-$ ion. The other distinguishing features of these spectra are as follows. The spectrum of **3** (Fig. 2a) clearly shows the successive elimination of a galloyl group (m/z 769, 601, and 431), and since there is no peak arising from the deprotonated ellagic acid ion (m/z 301), it is obvious that **3** consists entirely of gallic acid ester groups. The ion at m/z 601 in the spectrum of **4** (Fig. 2b), indicating the presence of a gallagyl group (Chart 3), was diagnostic. On the other hand, in the case of **5** which possesses an open-chain form of glucose, the fragmentation hardly occurred (Fig. 2c), probably because the ester bonds are relatively

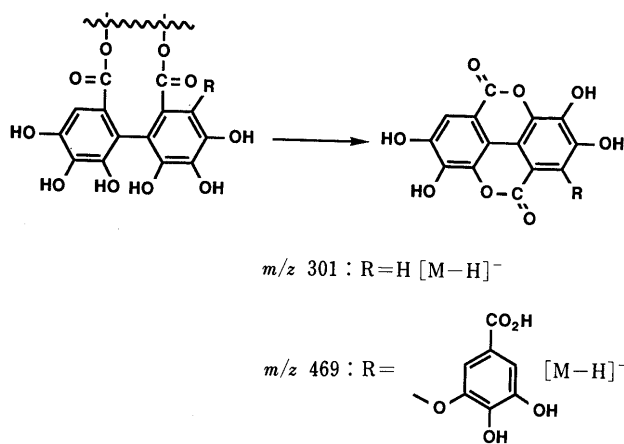


Chart 2

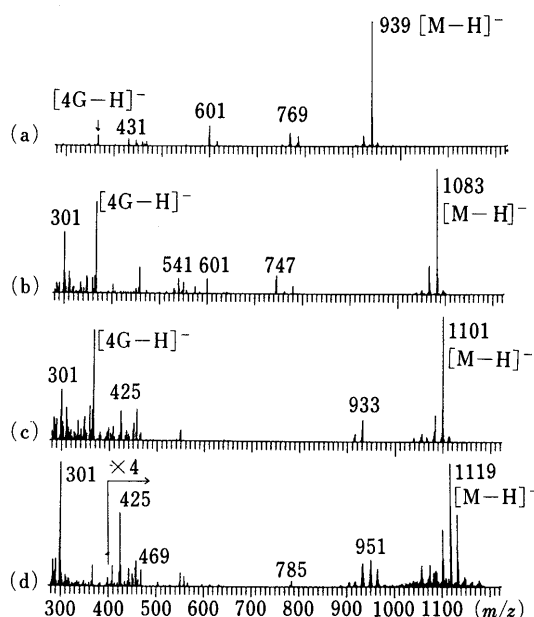


Fig. 2. The Negative Ion FAB Mass Spectra of (a) 1,2,3,4,6-Pentagalloyl Glucose, (b) Punicalagin, (c) Castavalonic Acid, and (d) Mallotusinic Acid with an HMPA-Glycerol Matrix
G, glycerol.

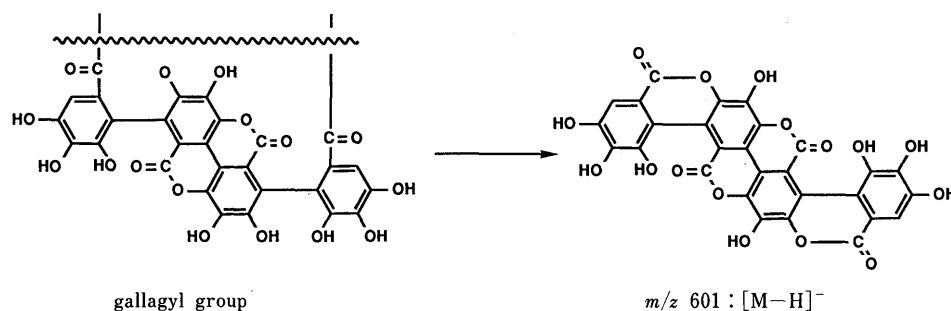


Chart 3

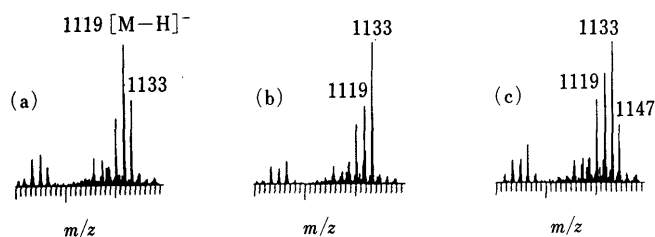


Fig. 3. The Negative Ion FAB Mass Spectra of Mallotusinic Acid

Sample preparations: (a) H_2O , (b) MeOH, and (c) EtOH.

rigid. The presence of a dehydrohexahydroxydiphenyl (DHHDP) group in **6** is readily recognized from the presence of the accompanying ion at m/z 1133 (Fig. 2d) which may be attributable to $[(M-OH)+OCH_3-H]^-$, probably formed by the replacement of a hydroxyl group with a methoxyl group. This phenomenon can be explained in terms of solvation by methanol used during the isolation, and further support for this was obtained by adding ethanol, which gave a relatively intense $[(M-OH)+OC_2H_5-H]^-$ peak at m/z 1147 (Fig. 3).

Analysis of the permethyl derivatives of tannins by positive ion FABMS with hydrophobic matrixes, e.g. *m*-nitrobenzyl alcohol, was also successful, but the method described here is indispensable to examine the original structure of tannins.

Experimental

The conditions for the measurements of negative ion FAB mass spectra were as follows. An aqueous solution of the sample was prepared at a concentration of ca. $1 \mu g/\mu l$, and the solution ($1 \mu l$) was mixed with $1 \mu l$ of

HMPA-glycerol (1:1, v/v) on a stainless-steel tip. Ionization was achieved by bombardment with a neutral xenon atom beam accelerated at 6 keV. The mass spectrometer employed was a JMS-DX300 (JEOL Ltd., Tokyo) equipped with a FAB ion source and interfaced to a JMA-3500 data processing system (JEOL Ltd., Tokyo). The mass range m/z 1–2000 was scanned in 10 s at an ion source accelerating potential of 1.5 kV and averaged intensities in decade scans were recorded.

Materials All the hydrolyzable tannins used in this experiment were isolated and structurally elucidated according to previous reports.⁷⁾ All chemicals were of analytical or reagent grade.

References and Notes

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