Mass Spectrometry of Selected Components of Biological Interest in Green Tea Extracts

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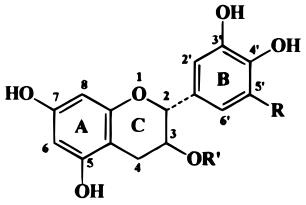
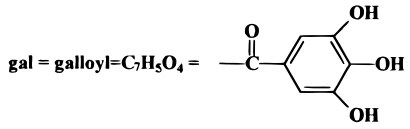
Mass spectrometric methods including EIMS, FABMS, and LC/ESIMS have been surveyed as tools for the detection of catechins in extracts of green tea (*Camellia sinensis*). EIMS provide both molecular weight and structure information, including epimer differentiation, on compounds **1**, **2**, and **4** and some structural information with compounds **5** and **6**. FABMS gives both molecular weight and structure information, by an retro-Diels−Alder mechanism, on all compounds. LC/ESIMS provides unambiguous molecular weight information on all compounds and some additional structural data are evident in the mass spectra of **5** and **6**. LC/ESIMS is, thus, shown to be an appropriate method for the direct analysis of crude extracts of green tea. The information obtained in this work will be of importance in future studies involving identification of the components of green tea and for characterization of synthetic analogues.

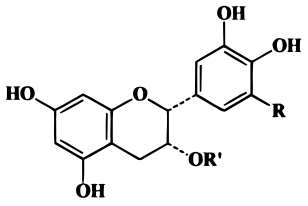
Considerable interest has been expressed in the biological activity of the components of green tea (*Camellia sinensis*,Theaceae), particularly in the antitumor and antioxidant properties of the polyphenolic fraction of the plant.[1] Recent studies from our laboratories have investigated the inhibitory effect of six green tea catechins and caffeine on the growth of four selected human tumor lines.[2] Pure (+)-catechin (C, **1**), (−)-epicatechin (EC, **2**), (+)-gallocatechin (GC, **3**), (−)-epigallocatechin (EGC, **4**), (−)-epicatechin gallate (ECG, **5**), and (−)-epigallocatechin gallate (EGCG, **6**), along with caffeine, were tested against MCF-7 breast carcinoma, HT-29 colon carcinoma, A-427 lung carcinoma, and UACC-375 melanoma for growth inhibitory effects. The most active of the catechins in these studies were **3**, **4**, and **6**. Further work has shown a significant antiphotocarcinogenic effect in BALB/cAnNHsd mice treated with a topical preparation of **6**.[3] Untreated mice had a skin cancer incidence of 96% at 28 weeks after exposure to UV irradiation; animals treated with 10 or 50 mg of **6** showed a decrease in skin cancer incidence to 62 and 39%, respectively, relative to the control group. As a result of these promising findings, we initiated studies to develop mass spectrometric methods to aid in the identification and structure elucidation of metabolites and synthetic analogues of these bioactive catechins. Thus, we have surveyed EIMS, FABMS, and LC/ESIMS methods for the analysis of reference samples of **1**−**6** and for analysis of **1**−**6** in complex mixtures, with the goal of applying these methods to the structure elucidation of metabolites or synthetic analogues of **1**−**6**.



1 (+) - Catechin (C), R=R'=H

3 (+) - Gallocatechin (GC), R=OH, R'=H





2 (-) - Epicatechin (EC), R=R'=H

4 (-) - Epigallocatechin (EGC), R=OH, R'=H

5 (-) - Epicatechin gallate (ECG), R=H, R'=gal

6 (-) - Epigallocatechin gallate (EGCG), R=OH, R'=gal

The EIMS of **1** and **2** and the permethylated derivative of **4** were first reported in 1968,[4] but no reports of the EIMS analysis of **3**−**6** have appeared in the literature. Of particular structural significance in the EIMS of flavan-3-ols is the occurrence of a retro-Diels−Alder (RDA) fragmentation, which allows for location of vari-

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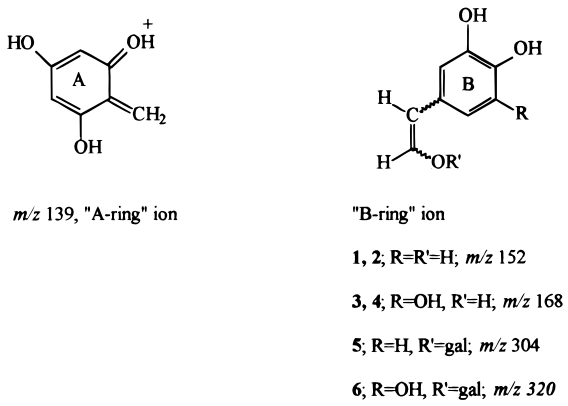


Figure 1. Structures of the ring A and ring B ions formed by the RDA mechanism.

ous substituents on either the A- or B-rings. The structures of these ions are shown in Figure 1.

A few reports have described the use of FABMS for the analysis of flavan-3-ols.[5−8] Liquid secondary ion mass spectrometry (LSIMS), a technique closely related to FABMS, has been reported to produce a protonated molecule ion at *m/z* 291 in the spectrum of **2**, which decomposes via the RDA to ions at *m/z* 139 and 152.[5] However, peaks at *m/z* 139 and 152 are present in the spectrum of the matrix used in the analysis, 3-nitrobenzyl alcohol (NBA).[6] Because the spectrum of **2** was not obtained in another matrix having different background ions, the occurrence of the RDA during FABMS of flavan-3-ols is not unambiguously established. In addition, NBA produces strong background ions in the region of the MH^+ ions of **1**−**4** and, thus, would be expected to mask, at least partially, the presence of these compounds. FABMS has also been used to characterize mixtures of green tea flavanols.[7] However, differentiation of epimeric pairs, for example, **1** from **2** or **3** from **4**, was not possible because no chromatographic separation was used. Also, no apparent attempt was made to identify structurally relevant fragment ions useful in the structure elucidation of the flavan-3-ols. One additional study describes the analysis of polyphenolic compounds from the sap of *Croton lechleri*.[8] The presence of **1**−**4**, and more complex dimers and trimers, in an extract of this plant was noted following chromatographic fractionation and analysis by FABMS and NMR. Each of these studies used a different matrix and no systematic evaluation of the effect of the matrix on the resulting FABMS mass spectra of the flavan-3-ols of interest has been conducted. No report of the use of LC/ESIMS for the identification, structure elucidation, or quantification of flavan-3-ols has yet appeared in the literature.

To optimize the mass spectrometric analysis of flavan-3-ols obtained from natural or synthetic sources, we have examined the EIMS, FABMS and ESIMS characteristics of a set of reference samples (i.e., **1**−**6**). In addition, LC/ESIMS was used for the analysis of a mixture of the reference samples, and the same technique was then applied to the direct analysis of a polyphenolic extract of green tea.

Results and Discussion

EIMS has previously been shown to provide molecular weight and structural information in the analysis of **1** and **2** and the permethylated derivative of **4**.[4] No reports have appeared describing the use of EIMS for the analysis of **4**, **5**, or **6**. To have a complete set of reference spectra for the catechins of interest, we have analyzed **1**−**6** using standard EIMS conditions for the generation of MS libraries. The mass spectra of **1** and **2** obtained in the present study show considerable variation in ion intensities from the previously reported data.[4] For example, the relative intensity of the $M^{\bullet+}$ ions in the spectra of **1** and **2** is considerably greater than values previously reported in the literature.[4] This difference is significant because, during a library search, the spectrum of a known compound may be mismatched or overlooked because of significant differences in ion intensities. The EIMS analysis of **1** and **2** also shows an epimer-specific loss of H_2O, with the intensity of the M-18 peak being <1% in the spectrum of **1**, but of 15% relative intensity in the spectrum of **2**. Thus, the ability to distinguish epimers of the flavan-3-ols is, at least in some cases, possible. Additional epimer pairs will need to be studied to determine the generality of this observation.

The EIMS spectrum of **4** shows all of the characteristic ions observed in the spectra of **1** and **2** with the $M^{\bullet+}$ and M-18 being useful for molecular weight assignment. [See Experimental Section for *m/z* (RI) data for **1**, **2**, and **4**.] The A-ring fragment (*m/z* 139), produced via an RDA mechanism, is the base peak of the spectrum, and the conjugate RDA fragment representing the B-ring is observed at *m/z* 168 (58% RI). The addition of the third OH group to the B-ring does not influence the fragmentation to any significant degree. This is fortuitous because the substitution pattern in the A- and B-rings is known to greatly affect the fragmentation pattern of flavonoid compounds.[9]

Transformation of the OH-3 group to a galloyl ester changes the EIMS dramatically. First, no molecular ion is observed for the intact molecule, a result of the more polar nature of **5** relative to **1**, **2**, and **4**. The peak at highest mass appears at *m/z* 304 (5%) and represents charge retention by the RDA product containing both the B-ring and the galloyl ester group. The conjugate fragment containing the A-ring is represented by the RDA+1 ion at *m/z* 139 (45%). Second, the most prominent ion in the mass region above *m/z* 200 in the spectrum of **5** is at *m/z* 272 (28%). Formation of this ion can proceed from the $M^{\bullet+}$ ion by a McLafferty rearrangement involving the C2 hydrogen with inductive cleavage of the C3−O bond, followed by elimination of gallic acid. Peaks related to retention of charge by the galloyl moiety dominate the remainder of the spectrum of 5. An ion at *m/z* 170 (98%) represents the intact gallic acid group, which, in turn, produces the base peak of the spectrum at *m/z* 153 by elimination of an OH radical. Thus, although no molecular ion is observed in the EIMS of **5**, a number of structurally diagnostic ions are observed, which permits identification of the A- and B-ring portions of the molecule, along with ions related to the galloyl group.

The EIMS of **6** is dominated by ions related to the galloyl function at *m/z* 170, 153, and 126 (loss of CO_2 from *m/z* 170). The only ion indicating the flavanol nucleus is the A-ring RDA fragment at *m/z* 139 (38%);

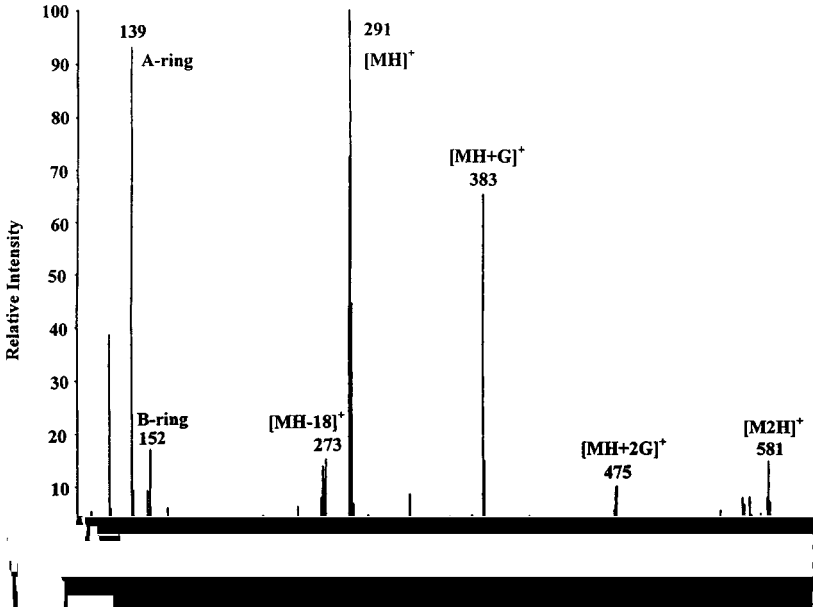


Figure 2. FABMS of **1** obtained using glycerol as the matrix. The spectrum shown has been background subtracted to remove most of the matrix-related ions.

no indication of the conjugate RDA fragment containing the B-ring is observed.

In summary, EIMS provides both molecular weight and structural information on **1**, **2**, and **4**, and some ions with structural relevance are present in the spectra of **5** and **6**, but molecular ions are not observed in the latter two compounds. EIMS does show an epimer-specific elimination of H_2O, at least in comparing the spectra of **1** and **2**, but additional studies would be needed to determine the generality of the reaction.

A systematic study of matrix effects for the FABMS analysis of flavan-3-ols is important because the quality of the resulting spectrum can be significantly influenced by the selection of matrix.[11] For example, the sensitivity for detection of a specific compound, the presence or absence of background ions that may mask sample related ions, the intensity of molecular-weight-related and structurally significant ions, and even the formation of specific ions during analysis can be influenced by the selection of the matrix.

Ions of structural importance in the FABMS of **1** and **2** include the MH^+ ion at *m/z* 291 and the RDA fragments containing the A- and B-ring fragments. The commonly used FAB matrix 3-nitrobenzyl alcohol (NBA), while being a versatile solvent,[6] has intense ions at *m/z* 307, 189, 154, and 136, which have the potential of masking sample related peaks in the spectra of compounds **1**−**6**. For this reason, glycerol (G) and thioglycerol (TG), two widely used FAB matrixes,[10] were selected as solvents for the analysis of the flavan-3-ols of interest in this study.

Glycerol provided clear MH^+ ions, along with expected G adducts, for each of the compounds **1**−**6**. An example of a typical FABMS obtained using G as the matrix is that of **1**, shown in Figure 2. The operation of the RDA is evident by the presence of ions at *m/z* 139 and 152, which represent the A- and B-ring fragments, respectively. The *m/z* 139 ion is present in the FABMS of all of the compounds **1**−**6** and provides information concerning the substituents of the A-ring. The conjugate RDA fragment representing the B-ring is present only in the spectra of **1** and **2**; analogues containing an additional OH group in the B-ring (i.e., **3**, **4**, and **6**) or the galloyl moiety (i.e., **5** and **6**) do not show the B-ring ion. A disadvantage of G as the matrix is that the spectra must be background subtracted to be useful, because of the intensity of the matrix ions. Prior to subtraction the spectra of all compounds are weak; however, following subtraction, the MH^+ ion becomes the base peak in the spectra of **1**, **2**, and **4**−**6**; compound **3** displays the *m/z* 139 ion as the base peak of its spectrum. In an effort to obtain more intense ions providing clear molecular weight and structural information, TG was examined as an alternative matrix for the FAB analysis of the flavan-3-ols.

Although TG produces a complex background spectrum, none of the matrix ions are coincident with sample related ions of the flavan-3-ols of interest. Compounds **1**, **2**, and **4**−**6** all display MH^+ ions with an intensity of 50% or greater, with no background subtraction being necessary to observe a "clean" spectrum. The spectrum of **3** was, in general, weak, and the MH^+ ion had a relative intensity of only 17%. The RDA reaction producing the *m/z* 139 ion gives the base peak in the spectra of **2** and **4** and is a strong ion (65% RI) in the spectrum of **6**. This ion however, is not present in the spectra of **1**, **3**, and **5**, and the conjugate fragment containing the B-ring is not observed in any of the samples. Compounds containing the galloyl ester also display a strong peak at $MH^+ - 170$ for loss of the gallic acid moiety, which is the base peak in the spectrum of **6** (See Figure 3); this ion is not observed when G is used as the matrix. Thus, FABMS obtained using TG as the matrix provide evidence for the presence of the galloyl ester function, but the RDA fragments are not as abundant as when G is used as the matrix. On the other hand, the intensity of the MH^+ ions is considerably greater in the TG spectra, and the molecular-weight-related ions are immediately obvious, without the need for background subtraction, as is the case when G is used as the matrix.

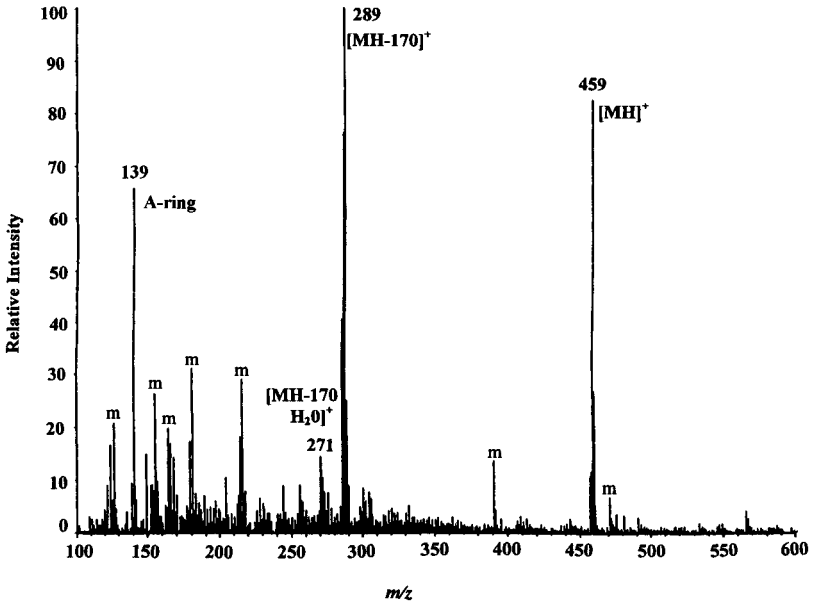


Figure 3. FABMS of **6** acquired using thioglycerol as the matrix (m = matrix ion).

Table 1. Major Ions of Interest in the FABMS of 1−6

glycerol (G)[a] cmpd	Ion A	Ion B	MH^+	$MH^+ + G$	other
C (**1**)	139 (91%)	152 (16%)	291 (100%)	383 (66%)	*m/z* 581 (M_2H^+, 16%)
					m/z 272 (M − H_2O, 14%)
EC (**2**)	139 (43%)	152 (24%)	291 (100%)	383 (20%)	*m/z* 272 (M − H_2O, 10%)
GC (**3**)	139 (100%)		307 (38%)		
EGC (**4**)	139 (55%)		307 (67%)	399 (100%)	*m/z* 491 (MH + 2G, 77%)
ECG (**5**)	139 (66%)		443 (100%)	535 (76%)	
ECGC (**6**)	139 (15%)		459 (76%)	551 (37%)	
thioglycerol					
1			291 (37%)		*m/z* 272 (M − H_2O, 15%)
2	139 (100%)		291 (90%)		*m/z* 272 (M − H_2O, 10%)
3			307 (17%)		*m/z* 289 (M − H_2O, 10%)
4	139 (100%)		307 (51%)		*m/z* 289 (M − H_2O, 7%)
					m/z 613 (M_2H^+, 4%)
					m/z 920 (M_3H^+, 1%)
5			443 (100%)		*m/z* 273 (MH^+ − 170, 68%)
6	139 (65%)		459 (83%)	565 (4%)	*m/z* 289 (MH^+ − 170, 100%)

[a] After background subtraction.

Negative ion FABMS [(−)FABMS] were also obtained on compounds **1**, **2**, and **4**−**6** using TG as the matrix. Compared to the (+) FABMS, the (−) FABMS provides little structural information, other than confirming the molecular weight of the sample. For example, the (−) FABMS of **6** shows only the $M_2 - H^-$ ion at *m/z* 915 (30%) and the $M - H^-$ ion at *m/z* 457; no indication of the RDA fragments or the loss of the galloyl group is evident.

In summary, FABMS provides a means of obtaining molecular weight and some structural information on all of the reference compounds examined and is, thus, an important tool for the analysis of the more polar analogues of the flavan-3-ols (e.g., **5** and **6**). The matrix providing the clearest molecular weight information was TG, although spectra obtained using G gave more structural information. NBA should be avoided as a matrix for the analysis of this compound class because of the presence of strong matrix-related ions at *m/z* values close to expected sample related ions. Ions of molecular weight and structural importance in the FABMS of **1**−**6** are shown in Table 1.

The use of LC/ESIMS methods for the analysis of complex mixtures of various classes of natural products has been recently reported,[12] but these methods have not been applied to the analysis of the flavan-3-ol class of compounds. We have, therefore, examined the LC/ESIMS behavior of compounds **1**−**6** as individual compounds and as a mixture of **1**−**6** and applied these methods to the analysis of a polyphenolic extract of *C. sinensis*.

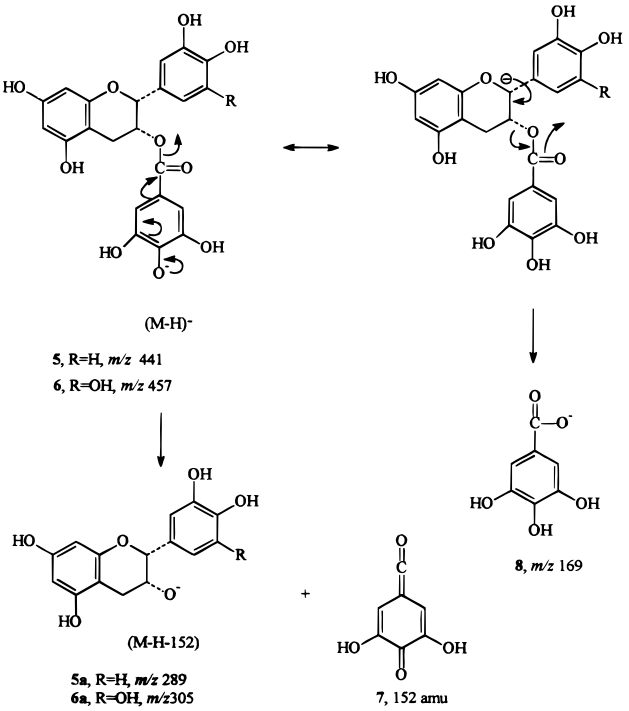
Initial experiments using LC/ESIMS were performed using positive ion detection; however, MH^+ ion intensities were very low using the standard conditions of analysis. Efforts to optimize the conditions for positive ion detection were not performed, and the negative ion detection mode was employed in these studies.

Analysis of the individual model compounds **1**−**6** using ESI provided very simple spectra with none of the RDA or other structurally important fragments being observed, in contrast to the more extensive structural information provided by EIMS or FABMS. The $[M - H]^-$ ion is the base peak in the ESIMS of each of the compounds **1**−**6**, which, along with other ions, provides unambiguous molecular weight information. Although no structurally relevant fragment ions are observed in the ESIMS of **1**−**4**, compounds **5** and **6** produce an $[M - H - 152]^-$ ion (**5a** and **6a**, see Scheme 1) indicating

Table 2. Ions of Structural Significance and Retention Time Data Observed in the (−) LC/ESIMS Analysis of 1−6

cmpd	RT [UV/MS][13]	(MH^-)	$(M - 2H + Na)^-$	other
C, **1**	4:14/4:24	289 (100)	311 (11)	
EC, **2**	4:45/4:56	289 (100)	311 (3)	
GC, **3**	2:44/2:54	305 (100)		
EGC, **4**	3:42/3:54	305 (100)	327 (2)	*m/z* 611 (2M − H)
ECG, **5**	5:54/6:03	441 (100)	463 (3)	*m/z* 289 (M − 152, 16%); 883 (2M − H), 169 (18%)
EGCG, **6**	4:56/5:06	457 (100)	479 (44)	*m/z* 169 (98%); 305 (M − H − 152; 24%); 915 (2M − H; 4%)

Scheme 1. Proposed mechanisms for the formation of the [M − H − 152]− (**5a**, **6a**) and *m/z* 169 (**8**) ions observed in the (−) ESIMS of **5** and **6**.



the presence of the galloyl ester. The ions observed in the (−) ESIMS of **1**−**6**, which contain molecular weight or structural information, are summarized in Table 2, along with retention time data for the LC/MS conditions used in these experiments.

After the analysis of each of the individual reference compounds, a synthetic mixture of **1**−**6** was prepared to determine whether the individual retention times remained constant in a complex mixture. No change was noted in the retention times determined for **1**−**6** individually or in the mixture.

Although ESIMS lack the structural detail observed in the EIMS, and to a more limited extent the FABMS, this ionization method is ideally suited to the analysis of complex mixtures in which the combination of retention time coupled with molecular weight information can be used for identification of known compounds or for the detection of unknowns. To illustrate such an application, a polyphenolic extract of green tea (*C. sinensis*) was subjected to the same analytical procedure as was used for the analysis of the reference sample mixture. The results of this analysis are shown in Figure 4. Ion profiles for each of the $[M - H]^-$ ions of compounds **1**−**6** are plotted and retention times correlated with the individual components.[13] The *m/z* 289 ion chromatogram represents **1** and **2** and shows maxima at the proper retention times for these compounds. In addition, a third component is seen whose maximum corresponds to the retention time of **5**. As mentioned earlier, **5** (and **6**) expels the galloyl group from the $[M - H]^-$ ion to produce the $[M - H - 152]^-$ ion, which, in the case of **5**, falls at *m/z* 289, the same mass as the $[M - H]^-$ ion for **1** and **2**. A proposed mechanism for the elimination of the galloyl function

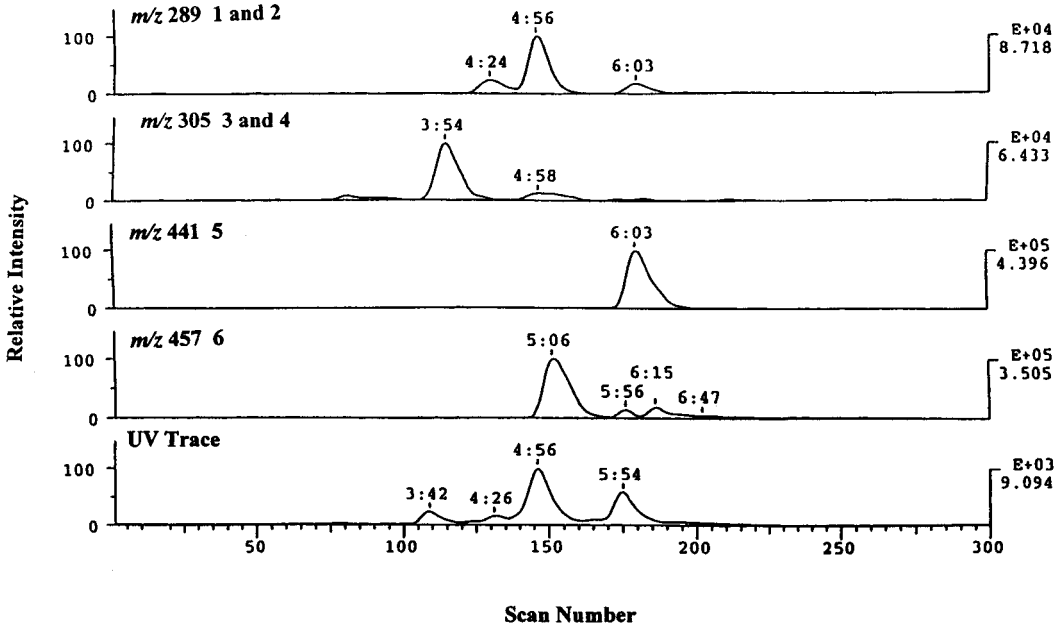


Figure 4. UV chromatogram and selected ion plots of the $[M - H]^-$ ions of compounds **1**−**6.** The data in this figure were obtained during the analysis of a crude extract of green tea (*Camellia sinensis*) leaves. The presence of unidentified, minor components is indicated by maxima in the ion plots that do not correspond in retention time to that of the reference materials.

is shown in Scheme 1. Thus, all three maxima in the *m/z* 289 ion chromatogram are identified.

Analysis of the *m/z* 305 ion chromatogram is more complex. The early, weak maximum centered approximately at scan number 60 is assigned as **3** based on retention time and an $[M - H]^-$ ion at *m/z* 305, and the component with a maximum at time 3:54 represents **4**. The broad peak centered at 4:58 is at least partially explained as arising from **6** by the loss of the galloyl function to form the $[M - H - 152]^-$ ion through the same mechanism by which **5** produces the *m/z* 289 ion (Scheme 1). In addition, at least three components display maxima (5:16, 6:15, and a later-eluting component) in the *m/z* 305 ion chromatogram that have retention times and $[M - H]^-$ ions different from any of the reference materials. The presence of these unknown compounds is also indicated in the *m/z* 457 ion trace representing **6**, where the ion intensity is greater and the presence clearer. These compounds are, at present, unidentified, and studies are underway to apply MS/MS techniques to identify these components.

An estimate as to the relative concentrations of the flavan-3-ols present in the green tea extract would be of value in comparing the amounts of the various catechins in different teas or in samples of teas extracted at different times. The information in Figure 4[13] can be used for both identification and relative quantitation of the components present in the polyphenolic extract. The ion chromatograms can be used to determine the molecular weights of the various components, and the UV data can be used to determine the relative concentrations of the components. Ranked in order of highest amount is **6** with a relative concentration (RC) of 1. Next in abundance is **5** with an RC of 0.52, followed by **2** (RC = 0.38), **4** (RC = 0.15), **1** (RC = 0.06), and **3** (RC = 0.02). These relative concentration values agree very well with the literature.[14] Thus, analysis of the polyphenolic extract chromatograms and mass spectral data could be helpful in optimizing the harvest of the desired compounds from green tea and in comparing various teas for their content of **1**−**6**.

In conclusion, each of the mass spectrometric methods has a role to play in the rapid analysis of crude plant extracts for the presence of known compounds of interest and in the structure elucidation of natural or synthetic analogues of the flavan-3-ols. LC/ESIMS has been shown to be an important tool for determination of the molecular weight of known components and for detection of possible analogues present in a polyphenolic extract of green tea. However, EIMS, FABMS, and MS/MS techniques are also needed to provide a greater amount of structural information than is provided by normal scan ESIMS data. The greatest amount of structural information is provided by EIMS, but the technique is limited to analysis of compounds of lower molecular weight. FABMS provides both molecular weight and some structural information, but selection of matrix is critical to obtaining ion currents of sufficient intensity to permit identification of the MH^+ ions without the necessity of background subtraction; best results in the FABMS analysis of the flavan-3-ols are obtained using TG as the matrix. The LC/ESIMS results of this preliminary study are currently being extended to include the use of MS/MS for the structure determination of minor components present in the polyphenolic extract of green tea.

Experimental Section

General Experimental Conditions. EIMS were obtained using a Finnigan MAT90 operating under the following conditions: ionization energy, 70 eV; source temperature, 250 °C; the mass range from 40 to 1000 Da was scanned at 2 s/decade with data being acquired, processed, and stored using a US Design Micro VIP 11/73 computer. Samples were introduced using direct probe insertion, the probe being heated from ambient temperature to 300 °C in 3 min. Spectra were averaged using 10 scans around the peak maximum.

FABMS were acquired using a modified Varian MAT 311A (AMD Intectra, Harpstedt, Germany). Data acquisition and instrument control were accomplished using two SAN 68K computers (KWS Computersysteme GmbH, Ettlingen, Germany) and software developed by AMD Intectra. Ionization involved a 1.6-mA primary beam of Cs^+ ions accelerated to 7 kV above source potential. Other instrumental parameters included: source temperature, 35 °C; accelerating voltage, 6 kV; resolution, 1000. Samples were premixed with the matrix (glycerol, thioglycerol, or 3-nitrobenzyl alcohol) and approximately 1 μL of the sample-matrix mixture applied to the stainless steel target. Ten spectra were averaged to produce the data described herein. The spectra acquired using glycerol as the matrix required background subtraction to produce usable data because of the intense signals from the matrix.

LC/ESIMS experiments were performed on a Finnigan TSQ 7000 instrument using the following conditions: negative ion detection mode, centroiding mode, multiplier at 1600 keV, 1000 amu/sec, source at 4.5 kV, sheath gas at 70 psi, auxiliary gas at 25 psi, capillary temperature at 220 °C, and UV detection at 220 nm. Liquid chromatographic conditions utilized an HP 1090 HPLC with a Zorbax SB C^{18} column (2.1 × 150 mm) and a mobile gradient of 10% MeCN/90% 2 mM ammonium acetate, pH 5, to 90%/10% over 15 min and holding for 5 min at a flow rate of 0.3 mL/min. ^1H and ^{13}C data were acquired on a Varian Unity 300 (300 MHz/75 MHz).

Plant Material. The plant material was provided by the Royal Estates Tea Company, a Division of Thomas J. Lipton, Co., Englewood Cliffs, NJ. The green tea blend was labeled "Green Research Standard".

Extraction and Isolation. Dried, green tea leaves (1 kg) were ground to a fine powder and macerated four times with EtOH (95%)−H_2O (1:1). The extracts were filtered, combined, and dried at room temperature. The crude extract (307 g) was redissolved in H_2O and the aqueous phase was exhaustively extracted with EtOAc. After separation of the phases, evaporation of the EtOAc provided 141 g of crude extract of green tea polyphenols that was used in the LC/ESIMS experiment and is referred to as polyphenolic extract in the text. A portion of the green tea extract (65 g) was chromatographed on a Sephadex LH-20 column (78 × 10 cm)(Pharmacia, Uppsala, Sweden) with 95% EtOH to yield five different semi-purified fractions containing the catechin derivatives and caffeine.

Purification of 2−6. Crude fractions of **2** and **5** were purified separately using a Si gel column (75 ×

5.5 cm)(Si gel 60, 0.05−0.2 mm; Macherey−Nagel, Duren, Germany) washed with CH_2Cl_2−EtOAc−HOAc (40:60:1). The fraction containing **3** and **4** was purified in a similar manner except that the mobile phase was CH_2Cl_2−$(CH_3)_2O$−HOAc (60:40:1). Column fractions containing each of the purified compounds were evaporated *in vacuo*. The obtained **2**−**5** were dissolved in a small amount of EtOAc, precipitated with CH_2Cl_2, and filtered through a fritted funnel. Semi-purified **6** was stirred with H_2O overnight at room temperature and filtered through a fritted funnel. The powder was washed repeatedly with H_2O to obtain pure **6**.

(+)-Catechin (1): purchased as (+)-catechin hydrate from Aldrich (Milwaukee, WI); EIMS: *m*/*z* 290 ($M^{\bullet+}$, 20%), *m*/*z* 272 (M − H_2O, 1%), *m*/*z* 152 (B-ring, 47%), *m*/*z* 139 (A-ring, 100%), *m*/*z* 123 (73%), *m*/*z* 110 (18%), *m*/*z* 77 (26%), 69 (41%).

Compounds **2**−**6**. The structural identity of **2**−**6** was established using spectral data, including MS and ^1H and ^{13}C NMR, and comparison of these data with those reported in the literature.[15−18] Copies of the spectral data can be requested from the authors.

Compound 2: EIMS 290 ($M^{\bullet+}$, 62), 272 (M − H_2O, 18), 152 (B-ring, 75), 139 (A-ring, 100), 123 (A-ring, 80), 110 (39), 77 (34), 69 (41).

Compound 4: EIMS 306 ($M^{\bullet+}$, 38), 288 (M − H_2O, 15), 168 (B-ring, 54), 139 (A-ring, 100), 126 (31), 110 (10), 97 (10), 69 (34).

Compound 5: EIMS 274 (M − 168, 25), 272 (M − 170, 32), 171 (22), 170 (gallic acid, 100), 153 (170 − $OH^{\bullet}$, 98), 139 (A-ring, 45), 126 (64).

Compound 6: EIMS 290 (M − 168, 22), 171 (30), 170 (gallic acid, 100), 153 (170 − $OH^{\bullet}$, 92), 152 (170 − H_2O, 40), 139 (A-ring, 35), 126(72), 125 (52).

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