JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Atmospheric Pressure Ionization LC-MS-MS Determination of Urushiol Congeners

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This paper describes atmospheric pressure ionization (API) LC-MS-MS determination of urushiols, 3-*n*-alkenyl- and -alkyl-substituted catechols responsible for poison oak dermatitis. Urushiol was isolated from Western poison oak according to the method of Elsohly et al. (*1*) (*J. Nat. Prod.* **1982**, *45*, 532−538)—the purified preparation contained C_{17}- and C_{15}-substituted urushiols with zero, one, two, and three double bonds as determined from GC-MS analysis of trimethylsilyl derivatives. Urushiol mixtures were separated on a C_{18} reversed phase HPLC column with a methanol−water gradient with urushiols eluting in 100% methanol. Atmospheric pressure chemical ionization (APCI) produced primarily $[M - H]^-$ and MH^+ molecule ions. Electrospray ionization (ESI) yielded $[M - H]^-$ and adduct ions including $[M + Cl]^-$. Daughter ions of $[M - H]^-$ included quinoid radical anions ($[M - H - H_2]^-$ and m/z 122^-) and a benzofuran phenate (m/z 135^-). A suite of hydrocarbon fragments were produced by collision-induced dissociation of MH^+ directly or via an intermediate $[MH - H_2O]^+$ daughter ion. Six urushiol congeners, one not previously reported in poison oak, were determined by negative ion API-LC-MS-MS with detection limits of ~8 pg/μL (ESI) and ~800 pg/μL (APCI). API-LC-MS-MS was used to determine urushiol in surface wipes, air samples, and plant materials.

KEYWORDS: Urushiol congeners; poison oak allergens; API-LC-MS-MS analysis

INTRODUCTION

Urushiols are lipophilic allergens present in plants of the Anacardiaceae family including Western poison oak (*Toxicodendron diversilobum*), Eastern poison oak (*T. pubescens*), poison ivies (*T. rydbergii* and *T. radicans*), poison sumac (*T. vernix*), and the Asiatic lacquer tree (*T. vernicifluum*). Urushiols cause irritation, inflammation, and blistering in sensitive individuals, and the associated recurrent contact dermatitis is a major occupational health problem in workers employed outdoors (*2*).

As many as 210 million people in the United States are vulnerable to urushiol (*3*). Sensitivity studies have established that 40% of human subjects respond to doses of 2 μg or less, about the amount of urushiol oil received on brushing against a single plant (*4*). Although a very rare complication, renal damage (nephrotic syndrome) has been associated with poison oak dermatitis (*5*).

Naturally occurring urushiols are catechol derivatives with various alkyl or alkenyl substituents. The olefinic side chains typically have one, two, or three double bonds. Upon hydrogenation two skeletons are obtained, 3-pentadecylcatechol and 3-heptadecylcatechol (**Table 1**.). The congener distribution varies among the Anacardiaceae species. Poison oak urushiols are principally 3-heptadecylcatechols with two or three side-chain double bonds (*6*). The allergenicity of urushiols is a function of both the catechol ring and the side chain. Dimethyl ethers are inactive (*1*), and potency increases with side-chain unsaturation.

Urushiols are sensitive to oxidation and polymerization. Polymerization in lac (or shellac) formation is initiated by enzymatic oxidation (*7*), although urushiols autoxidize as well (*1*). Urushiol binds irreversibly and/or decomposes on chromatographic adsorbents such as alumina and silica gel (*8*). Despite their instability, decaying poison oak plants remain biologically active for many years, and smoke from burning plant debris also is highly allergenic.

GLC determination of urushiol was initially reported 40 years ago (*8*), and urushiols were first characterized by GC-MS in 1975 (*6*). GC analysis requires derivatization to stabilize the molecules and improve their chromatography. Urushiols have been determined directly by HPLC with UV (*9*), electrochemical (*7*), and refractive index detectors (*1*, *10*). On octadecylsilane (ODS) reversed phase columns urushiols are retained according to degree of unsaturation (*7*).

We report here the use of atmospheric pressure ionization (API) LC-MS for urushiol determination. Urushiols are not commercially available and were isolated from a plant source

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10.1021/jf011364t CCC: $22.00
Published on Web 03/01/2002

Table 1. Urushiol Structures of Poison Oaks and Poison Ivies

3-pentadecyl catechols			3-heptadecyl catechols		
double bonds	formula	monoisotopic mass	double bonds	formula	monoisotopic mass
0	$C_{21}H_{36}O_2$	320	0	$C_{23}H_{40}O_2$	348
1	$C_{21}H_{34}O_2$	318	1	$C_{23}H_{38}O_2$	346
2	$C_{21}H_{32}O_2$	316	2	$C_{23}H_{36}O_2$	344
3	$C_{21}H_{30}O_2$	314	3	$C_{23}H_{34}O_2$	342

and initially characterized by GC-MS. HPLC conditions were developed using a C_{18} reversed phase column and API-MS was examined in various MS modes. This research led to the development of a practical and quantitative LC-MS-MS method for determination of urushiols in surface wipes, air samples, and plant materials.

MATERIALS AND METHODS

Urushiol Isolation. Poison oak was extracted with solvent, and extracts were purified according to the method of Elsohly et al. (*1*). Briefly, plant material was cut into small pieces, weighed, and transferred to an Erlenmeyer flask with a stirring bar for extraction with 95% ethanol. Samples were immersed in solvent and stirred vigorously for 4 h. The solvent was decanted, filtered, and removed just-to-dryness on a rotary flash evaporator. The residue was dissolved in 25 mL of dichloromethane and washed with 3 × 15 mL of sodium chloride-saturated water. The organic layer was reduced to dryness giving a crude extract or subjected to further purification by column chromatography and liquid−liquid partitioning to obtain purified urushiol.

The dichloromethane extract was applied to a 1 × 15 cm silica gel 60 column and eluted by addition of a further 100 mL of dichloromethane. The eluent was reduced to dryness on a rotary evaporator, dissolved in 20 mL of hexane, and partitioned with 3 × 20 mL of acetonitrile; the combined acetonitrile phases were concentrated and stored in amber glass vials at 20 °C. Accordingly, a 7.1 g Western poison oak specimen yielded 320 mg of crude extract and 22 mg of purified urushiol.

Urushiol Trimethylsilyl (TMS) Derivatives. For GC-MS analysis urushiol was derivatized with bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) (*1*). Samples in 100 μL of acetonitrile were combined with 100 μL of Sylon BFT (BSTFA and TMCS, 99:1 v/v, Supelco, Bellefonte, PA) and 50 μL of dry pyridine in a miniature vial. Samples were heated in an 80 °C water bath for ∼30 min and, after cooling, 50 μL of the reaction mixture was diluted to 1 mL before analysis.

Gas Chromatography—Mass Spectrometry. GC-MS was used to characterize the urushiol composition of the plant extract. An HP 5973 mass spectrometer with a 6890 series gas chromatograph (Agilent, Wilmington, DE) operated in electron ionization (EI) mass scanning mode was used. TMS derivatives were separated on a 30 m × 0.25 mm (i.d.), 0.25 μm film HP-5MS capillary column (Agilent) using splitless injection and an oven temperature program. The GC-MS conditions were as follows: inlet temperature, 225 °C; transfer line temperature, 280 °C; initial oven temperature, 50 °C (held for 5 min); 15 °C/min ramp to 250 °C (held for 15 min); source temperature, 230 °C; scan range, 45−600 amu. The helium flow rate was 1 mL/min.

Liquid Chromatography—Mass Spectrometry. A Finnigan LCQ-Deca quadrupole ion trap (QIT) mass spectrometer (Finnigan MAT, San Jose, CA) equipped with both ESI and APCI probes was used. The HPLC was an HP 1100 (Agilent) equipped with an autosampler, solvent degasser, binary pump, heated/cooled column compartment, and in-line diode array detector (DAD). The mass spectrometer and HPLC inlet were controlled by Finnigan Corp. Xcalibur software (version 1.0, SR 1), which also was used for data acquisition and processing.

Compounds were introduced to the APCI sample tube or fused silica-lined ESI needle by syringe pump (ESI only) at 2 μL/min flow rates or via the 0.4 mL/min chromatographic mobile phase. The mass spectrometer was initially tuned and calibrated in ESI using a tuning mixture containing caffeine, a tripeptide, and a synthetic polymer mixture. For day-to-day LC-MS operation a milligram per milliliter metobromuron tuning solution in methanol/water (9:1, v/v) was used—the compound was introduced into the flowing mobile phase at 2 μL/min via a T-fitting. In both APCI and ESI operations the instrument was tuned on *m*/*z* 257 ($[M - H]^-$) or *m*/*z* 259 (MH^+) ions with all voltages, offsets, and lens settings determined by autotune software.

A 2.1 mm × 15 cm Supelcosil LC-18 column (Supelco) with 3 μm packing was used exclusively. Two solvent programs were employed. Solvent program 1, used for both APCI and ESI mass spectrometry, was a 14 min linear methanol/water gradient from 50 to 100% methanol with a uniform 0.4 mL/min flow rate. Solvent program 2 (ESI only) was a 14 min methanol/water gradient from 30 to 100% methanol with a 0.5 mL/min flow rate. The ESI spray voltage was 5 kV, the ESI heated capillary temperature was 350 °C, and the nitrogen sheath and auxiliary gas flows were 80 and 20 units, respectively. The mass spectrometer acquired for 18 min in centroid mass scanning mode from 175 to 400 amu after a 0.5 min start delay. The QIT mass analyzer was operated with automatic gain control with three total microscans and a 50 ms maximum injection time. The electron multiplier voltage was 880 V.

In APCI operation the vaporizer and capillary temperatures were 450 and 200 °C, respectively. Auxiliary and sheath gases were maintained at 20 and 80 units, respectively. The scan range in APCI mass scanning mode was *m*/*z* 100−650.

Surface Wipe Samples. Laboratory tissues (Kimwipes EX-L; Kimberly-Clark, Atlanta, GA) soaked with 2-propanol were used to wipe a demarcated 100 cm^2 surface. Tissues were transported at 0 °C in plastic jars, and urushiols were extracted in the laboratory by addition of 10 mL of 95% ethanol followed by 3 min of vigorous shaking. The solvent was decanted, centrifuged (5000 rpm, 5 min), filtered through an 0.45 μm polypropylene cartridge filter (Alltech No. 655106, Deerfield, IL), and reduced to ∼0.2 mL under a stream of dry nitrogen. The sample was adjusted to 1 mL with methanol and again filtered—the cartridge filter was rinsed with an additional milliliter of methanol, and the combined filtrate was reduced to 1 mL prior to analysis.

CAUTION. Poison oak, poison ivy, and related plant species and their extracts and derivatives contain potent allergens exhibiting delayed hypersensitivity and should be handled only by using appropriate protective clothing and respiratory protection or a fume hood. Dried plant materials retain biological potency for many years, and their combustion products are hazardous.

RESULTS AND DISCUSSION

GC-MS Characterization of Urushiols in Western Poison Oak. The crude plant extract was analyzed by GC-MS to verify urushiol content. Urushiol di-TMS derivatives each gave the trimethylsilyl ion at *m*/*z* 73, $Si(CH_3)_3^+$, as base peak followed in intensity by the molecular ion. Tropylium ions substituted with one or two $-O-Si(CH_3)_3$ groups were present at *m*/*z* 179 ($C_9H_{11}SiO_2$) and *m*/*z* 267 ($C_{13}H_{23}O_2Si_2$), respectively, as well as minor $[M - CH_3]^+$ ions. The TMS spectra agreed well with those in the literature (*1*), and the observed P + 1 and P + 2 isotopes were consistent with the molecular formulas.

The retention times increased with molecular weight on the HP-5MS column with C_{15} urushiols eluting as a group prior to C_{17} urushiols (**Table 2**). The approximate distribution of congeners was 50 (C_{17} triene):8 (C_{17} diene):5 (C_{15} saturated):1 (C_{15} monoene), assuming equivalent response factors.

The mass spectrum of the principal congener is shown in **Figure 1**. Previous studies have established the poison oak C_{17} dienes have double bonds in the 8- and 11-positions and that the C_{17} trienes have 8,11,14-unsaturation (*1*, *2*, *6*) as shown. All double bonds are reported to have the cis configuration.

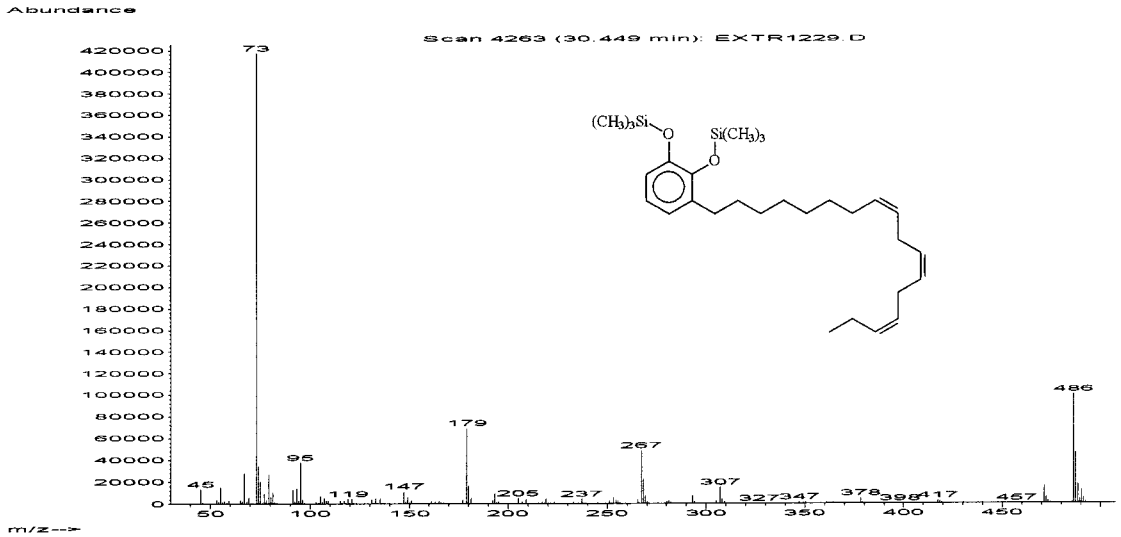

Figure 1. Electron ionization GC mass spectrum of principal urushiol from poison oak (as TMS derivative). The double-bond positions and configurations are based on the literature.

Table 2. Electron Ionization Mass Spectra of Urushiol TMS Derivatives

		relative abundance (%)					
compound	t_R (min)	*m/z* 73	*m/z* 179	*m/z* 207	*m/z* 267	$[M - CH_3]^+$	M^+ (*m/z*)
C_{15} monoene	25.1	100	19	19	7.4	<5	59 (*m/z* 462)
C_{15} saturated	25.5	100	16	6.7	9.6	7.7	75 (*m/z* 464)
C_{17} diene	30.2	100	18	<5	8.2	5.5	47 (*m/z* 488)
C_{17} triene	30.4	100	19	nd[a]	12	<5	25 (*m/z* 486)

[a] Not detected.

HPLC Separation. HPLC separation of the crude poison oak extract without derivatization revealed a number of UV-absorbing components. All of them were very nonpolar and eluted with a mobile phase composition of 100% methanol (**Figure 2**). Six compounds eluted between 14 and 16 min (solvent program 1), with the most abundant isomer at 14.5 min. Five of the six components had UV absorption spectra superimposeable with the spectrum shown in **Figure 2** with two absorption maxima, λ_{max} at ~205 nm and a second band at 276 nm. This is consistent with the fact that all of the urushiols share the 3-alkyl-1,2-benzenediol chromophore.

Two of the urushiols were resolved to baseline, t_R 14.5 min and 15.0 min, whereas the two late eluting urushiols were only partially resolved. The most polar urushiol, t_R 14.23 min, was incompletely resolved from an unknown with λ_{max} at 224 nm, which is seen as a leading shoulder in the chromatogram.

APCI Mass Spectrometry. Urushiol APCI spectra were generally single-ion spectra with prominent MH^+ or $[M - H]^-$ ions. The minor fragment ions (<5% relative abundance) included *m/z* 123^+, 163^+, and 177^+ and $[MH - H_2O]^+$ in positive ion and *m/z* 122^- and 135^- in negative ion. Although the positive ion signal intensity was ~20-fold greater, the noise level in the negative ion was <0.1%, resulting in a considerably better signal-to-noise ratio (S/N) in negative ion mode. As a result negative ion APCI was preferred and allowed determination of six urushiol congeners in the crude plant extract (**Table 3**).

Each of the urushiols was detected as a sharp chromatographic peak demonstrating negligible band broadening in the API mass spectrometer. The retention times appearing in the table do not correspond exactly because the diode array detector was upstream from the mass spectrometer, resulting in a slight offset, ~0.04 min. Moreover, there is experimental error as the data are based on chromatographic runs over a period of days; for example, the retention time of the main component was 14.60 ± 0.095 min ($n = 3$).

Small amounts of other congeners were tentatively identified in the extract including a C_{15} diene (*m/z* 315^-, t_R 14.33 min), a C_{15} triene (*m/z* 313^-, t_R 13.83 min), and a C_{17} pentaene (*m/z* 339^+, t_R14.29 and 14.42 min). APCI data revealed that three or more compounds coeluted in the cluster of peaks at t_R 14.23 min (**Figure 2**). This included compounds tentatively identified as the C_{17} tetraene and C_{17} pentaene and an unknown detected in both APCI modes at t_R 14.22 min (e.g., *m/z* 387^+ and 385^-).

Collision-Induced Dissociation. CID of urushiol MH^+ and $[M - H]^-$ ions generated by APCI was investigated as a means to enhance spectroscopic information and improve specificity. At low collision energies (e.g., <30 V) the olefinic urushiol MH^+ ions fragmented by loss of water. At higher collision energies (e.g., >35 V) the daughter ion spectra were very distinctive and indicative of a hydrocarbon with an envelope of ions spaced at 14 amu intervals (**Figure 3**) as in the series *m/z* 135, 149, 163, and 177 and *m/z* 189, 203, 217, 231, 245, and 259. The *m/z* 163 daughter ion was typically the base peak at high collision energies with the exception of the tetraene, where $[MH - H_2O]^+$ was prominent. This was the case with each of the four olefinic C_{17} congeners in the crude extract.

Proposed structures for the major daughter ions included the dihydroxytropylium ion (*m/z* 123) and a homologous series of alkyl-substituted benzopyran and/or benzofuran fragments that account for the hydrocarbon pattern (**Figure 4**). An MS^3 experiment of the C_{17} triene established that a hydrocarbon envelope also is formed by CID from the $[MH - H_2O]^+$ daughter ion as in the sequence $[MH]^+ > [MH - H_2O]^+ > 297^+, 283^+, 269^+, 255^+, 241^+, 227^+$.

Urushiol $[M - H]^-$ anions are more difficult to fragment. $[M - H]^-$ remained as the base peak even with a 40−50 V acceleration voltage. Two of the urushiol isomers lost hydrogen, and for the C_{17} triene $[M - H - H_2]^-$ was the daughter spectrum base peak over the range of 40−50 V (**Figure 5**). For all six urushiols determined by APCI, the *m/z* 122^- and 135^- daughter ions increased in abundance with collision energy, with the *m/z* 122^- fragment becoming the base peak. The hydrocarbon pattern was less prominent in negative

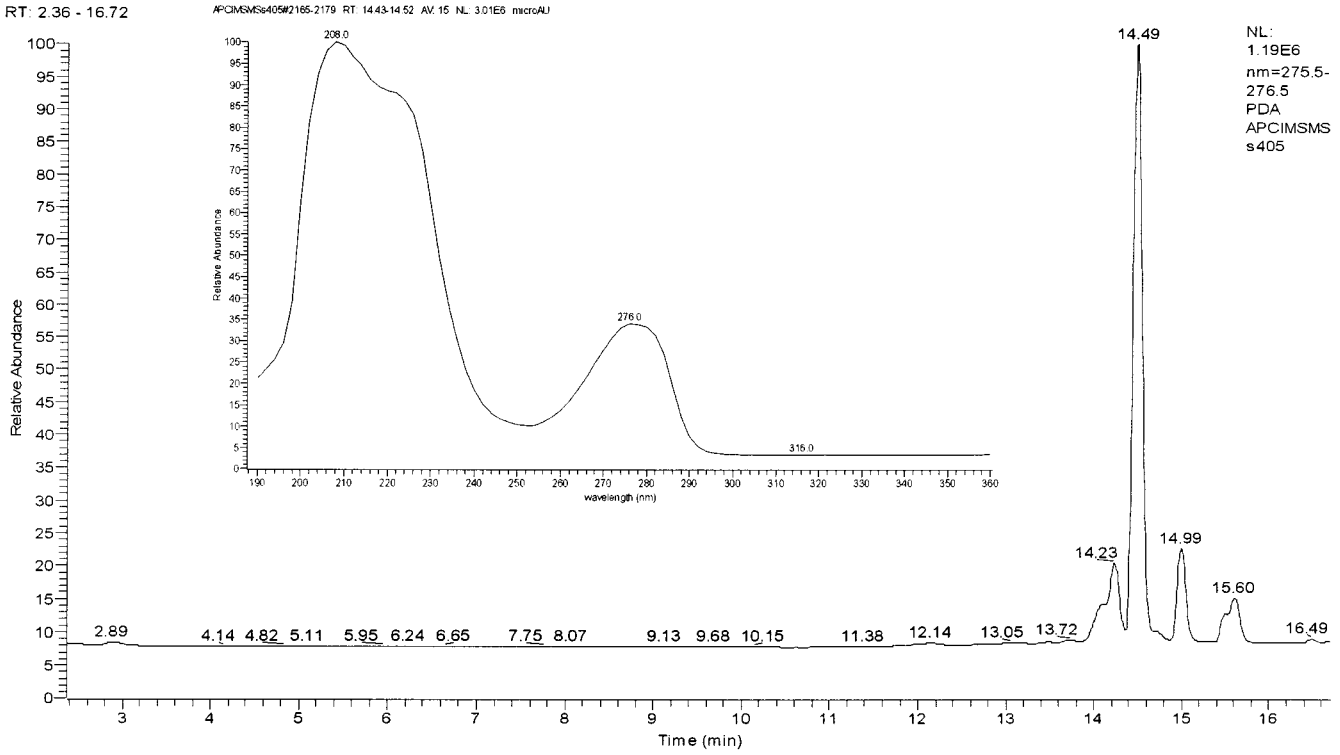

Figure 2. DAD chromatogram (276 nm window) of crude poison oak extract with DAD absorption spectrum (inset) for the most abundant urushiol congener at 14.49 min.

Table 3. Urushiol Retention Times and APCI Mass Spectra

		APCI MS			
urushiol congener	diode array t_R[a] (min)	positve ion base peak (MH^+)	t_R[a] (min)	negative ion base peak ($[M-H]^-$)	t_R[a] (min)
C_{17} tetraene	nd[b]	*m/z* 341	14.29, 14.51	*m/z* 339[a]	14.22, 14.39
C_{17} triene	14.49	*m/z* 343	14.66	*m/z* 341	14.59
C_{15} monoene	nd	nd	nd	*m/z* 317	14.88
C_{17} diene	14.99	*m/z* 345	15.16	*m/z* 343	15.11
C_{15} saturated	15.46[c]	nd	nd	*m/z* 319	15.65
C_{17} monoene	15.60[c]	*m/z* 347	15.82	*m/z* 345	15.77

[a] Solvent program 1. [b] Not detected. [c] Not resolved.

daughter ion spectra, although at very high collision energies (e.g., 55 V) the pattern was discernible for the C_{17} monoene.

Electrospray (ESI) Mass Spectrometry. Electrospray ionization also was effective for urushiols, particularly negative ion ESI. The instrument response in negative ESI was substantially greater than that in positive APCI, ~100-fold. At concentrations needed for diode array detection, ESI spectra showed signs of saturation with very broad and flat-topped ion chromatogram peaks. Reconstructed ion chromatograms for the minor components were sharp by comparison. Accordingly, urushiol was analyzed over a much lower concentration range in negative ESI (e.g., 8−4000 pg/μL) relative to APCI and positive ESI was not used.

Electrospray spectra of the partially resolved mixture components were obtained by background subtraction. The negative ion ESI spectra included additional adducts of masses 14, 35, and 45 amu. The 35 amu adduct was chloride, readily recognized by its ^{37}Cl isotope. The high mass adduct may be formate, $[M + HCOO]^-$, although the mobile phase did not contain formic acid. The $[M - H]^-$ ion always appeared as the base peak, and the chloride adduct was always present. Electrospray spectral features are summarized in **Table 4**.

In the crude extract the second urushiol congener eluting as a major band at 15.6 min gave the most intense electrospray signal—the least intense, ~$^1/_{20}$, was given by the earliest eluting congener (**Table 4**). The retention times do not correspond to those in **Table 3** because of differences in the solvent program. The DAD trace indicated that the component at t_R 14.49 min listed in **Table 3** is the same as the compound at t_R 15.60 min given in **Table 4**.

Chromatographic peaks for some of the components appeared to be split with adjacent peaks having nearly identical ESI mass spectra (**Figure 6**). The most polar urushiol, for example, eluted as two chromatographic peaks with retention times of 14.89 and 15.04 min and approximately equivalent areas. The leading peak or shoulder became less prominent with increasing retention time; for example, the ratios of the second peak to the first were 3:1, 13:1, and 14:1 for the urushiol compounds eluting at 15.4, 16.3, and 16.4 min, respectively.

The peak splitting may be due to the difference in polarity between the sample and the mobile phase at injection. Peak distortion is sometimes encountered with large injection volumes relative to the mobile phase flow rate. The phenomenon was

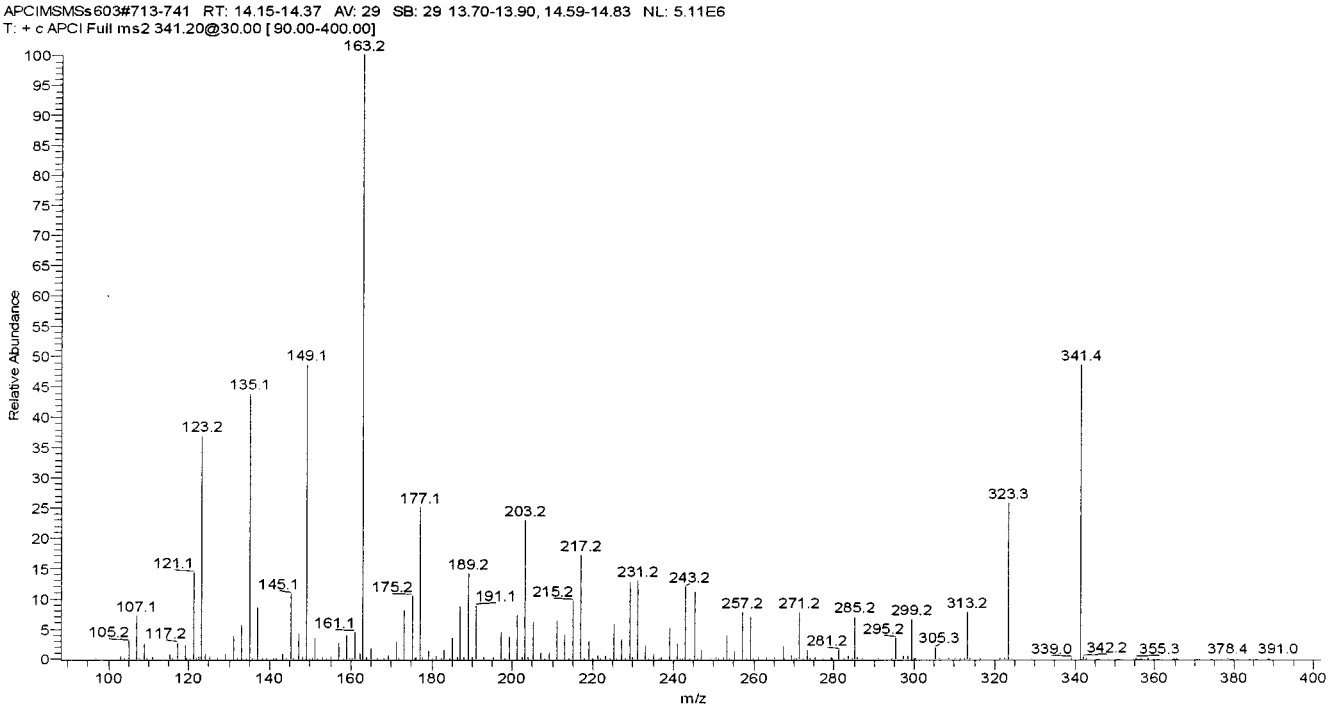

Figure 3. Daughter ions from fragmentation of C_{17} tetraene MH^+ (*m/z* 341$^+$) prepared by APCI (collision energy = 30 V).

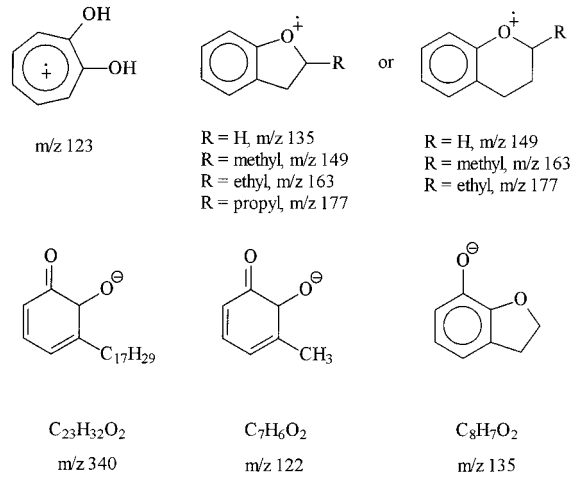

Figure 4. Proposed daughter ion fragments from CID of urushiol MH^+ and $[M - H]^-$ parent ions for both APCI and ESI mass spectrometry.

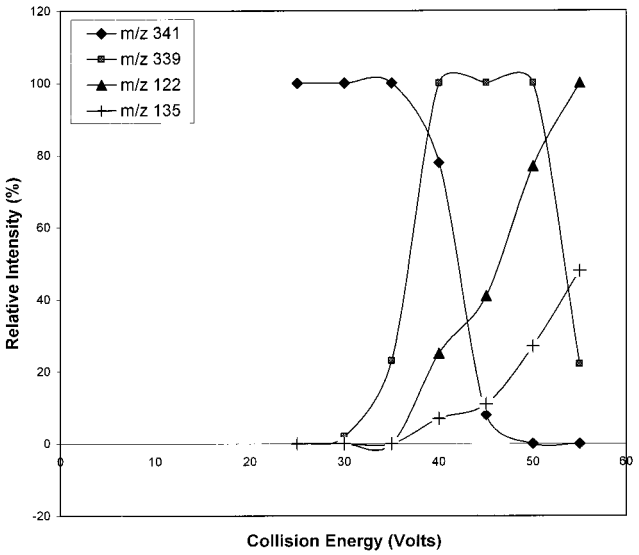

Figure 5. Effect of collision energy on daughter ion spectrum of the C_{17} triene $[M - H]^-$ parent ion at *m/z* 341$^-$.

more prominent in ESI analysis than in APCI or DAD, most likely due to differences in analysis concentration.

Repeated analysis of the standards in successive sample batches over a period of weeks revealed that the anomalous chromatographic behavior of the urushiols in ESI was not reproducible. Peak broadening has been previously reported in the separation of urushiols on high-efficiency ODS columns (*10*). An alternate explanation is that some of the urushiols exist as isomers that are resolved on the reversed phase column. For example, the Japanese lac tree contains both 8,11,14- and 8,11,13-substituted trienes, as well as thitsiol homologues with 1,2,4-ring substitution (*10*).

By any of the methods employed [diode array, GC-MS, APCI (positive and negative) and negative ESI] the C_{17} triene and C_{17} diene were the most abundant components in the Western poison oak extract. Although the urushiol structures identified are typical of those reported in the literature, two of the congeners are unique: the C_{15} saturated urushiol and the C_{17} tetraene. These were the least abundant components determined by LC-MS, and neither was detected by GC-MS. The C_{17} tetraene has not been reported previously in poison oak; however, it is a minor congener of Japanese lac (*10*).

Table 4. Urushiol Electrospray Spectra

congener	t_R (min)[a]	base peak ($[M - H]^-$)	$[M + Cl]^-$	relative area (%)
C_{17} tetraene	14.89	*m/z* 339	*m/z* 375	4.8
	15.04	*m/z* 339	*m/z* 375	3.0
C_{17} triene	15.29	*m/z* 341	*m/z* 377	35
	15.60	*m/z* 341	*m/z* 377	100
C_{17} diene	16.02	*m/z* 343	*m/z* 379	43
C_{15} saturated	16.39	*m/z* 319	*m/z* 355	15
C_{17} monoene	16.47	*m/z* 345	*m/z* 381	15

[a] Solvent program 2.

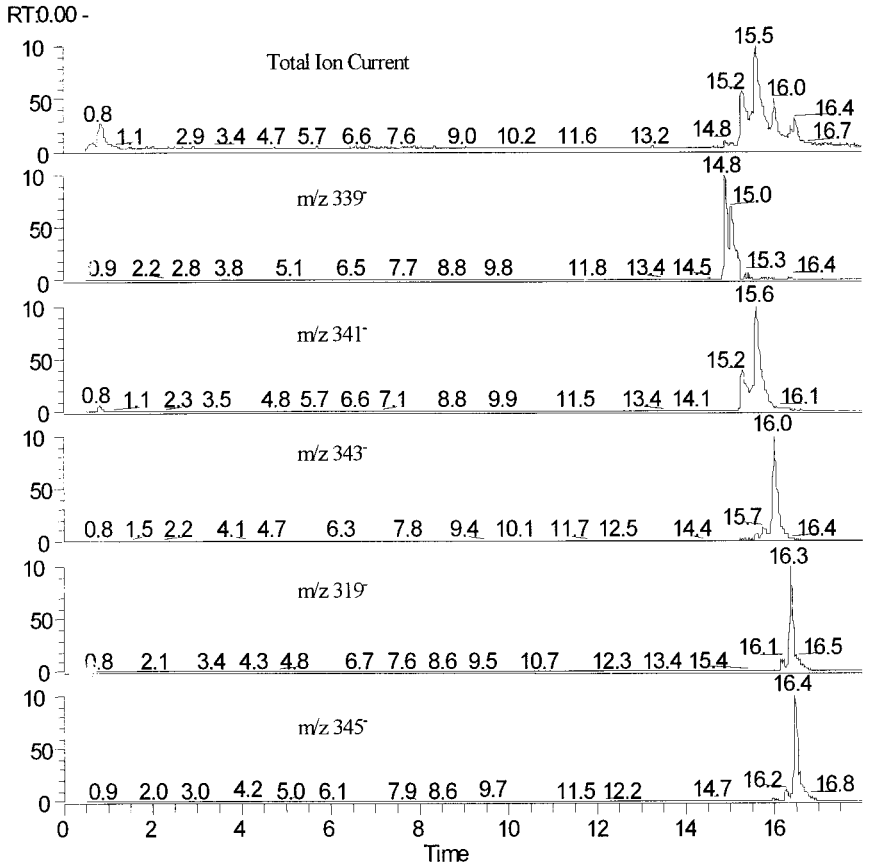

Figure 6. ESI chromatograms for urushiol congeners in Western poison oak: *m/z* 339$^-$ (C_{17} tetraene), *m/z* 341$^-$ (C_{17} triene), *m/z* 343$^-$ (C_{17} diene), *m/z* 319$^-$ (C_{15} saturated), and *m/z* 345$^-$ (C_{17} monoene).

CID of the C_{17} triene in ESI LC-MS-MS was equivalent to CID observed in APCI. The $[M - H]^-$ base peak (*m/z* 341$^-$) subjected to successively higher collision energies fragmented completely at 52 V. At this energy *m/z* 339$^-$, 122$^-$, and 135$^-$ were the major daughter ions. As in APCI two of the daughter ions, *m/z* 122$^-$ and 135$^-$, are nonspecific, whereas the higher mass daughter ion formed by loss of H_2 is congener specific.

Optimization of Negative APCI LC-MS-MS Detection. The collision voltage was varied between 25 and 55 V to optimize LC-MS-MS responses to the olefinic C_{17} urushiols. Maximum signals were obtained at 40 V (tetraene, $339^- > 337^- + 122^-$), 45 V (triene, $341^- > 339^-, 122^-$; diene, $343^- > 122^- + 135^-$), and 50 V (monoene, $345^- > 122^- + 135^-$). When selected reaction monitoring (SRM) was based on both the $[M - H - H_2]^-$ and low-mass daughter ions, an intermediate voltage yielding strong signals for both was used.

Various QIT mass spectrometer scanning options were examined including sequences with both multiple scan segments and multiple scan events. The former approach requires setting up retention time windows for the analytes, whereas the latter continuously cycles through scan events capable of detecting each congener. The first approach is limited by the chromatographic separation (e.g., the number of analytes eluting in a given time window), whereas the multiple scan events needed to determine all six urushiols limit the number of data points acquired across each chromatographic peak. For multicongener analysis a practical approach involved about four segments for the major C_{17} olefins eluting between 14 and 16 min.

Instrument Calibration. *APCI.* Quantitation of the major poison oak congener, C_{17} triene, was examined. The compound was determined by MS-MS of the reaction $341^- > 339^- + 122^-$ at 45 V. The parent ion was isolated with a window of ±0.5 amu, and daughter ions were scanned over two ranges, *m/z* 112−132 and 329−349. Ten-point calibration of the mass spectrometer with standards spanning a three-decade concentration range (0.8−800 μg/mL) established a quadratic response. The fit of the line was very good across the range, and the accuracy at 1 μg/mL was ±20%.

Electrospray. As noted above, negative ESI mass spectrometry had lower detection limits than APCI, ∼100-fold lower. In ESI-LC-MS-MS the C_{17} triene was determined by monitoring daughters of the $[M - H]^-$ parent ion, *m/z* 341, with a collision energy of 52 V. In this case the entire daughter ion spectrum (*m/z* 100−400) was acquired, and the *m/z* 339 ion chromatogram was plotted—the *m/z* 122 and 135 daughter ions were used only for confirmation. In ESI the mass spectrometer response was quadratic over the range of 8−4000 ng/mL. Working ranges for quantitative analysis, thus, were different for each of the API analysis methods investigated. For comparison purposes calibration data for the two API methods are plotted with electron ionization GC-MS data (**Figure 7**).

Conclusion. API-LC mass spectrometers are beginning to appear in environmental laboratories because of their potential for the determination of high molecular weight, polar, and reactive substances. In the present study API-LC-MS-MS was useful for direct determination of reactive urushiol congeners. The traditional methods of urushiol analysis involve tedious conversion to TMS ethers that have sufficient volatility and stability for GC. Both ESI and APCI are sufficiently sensitive for direct determination of urushiol in the low parts per million concentration range relevant for toxicological, clinical, and environmental studies of these allergens.

APCI was used in both polarity modes, whereas ESI was substantially more sensitive with negative ion detection. CID

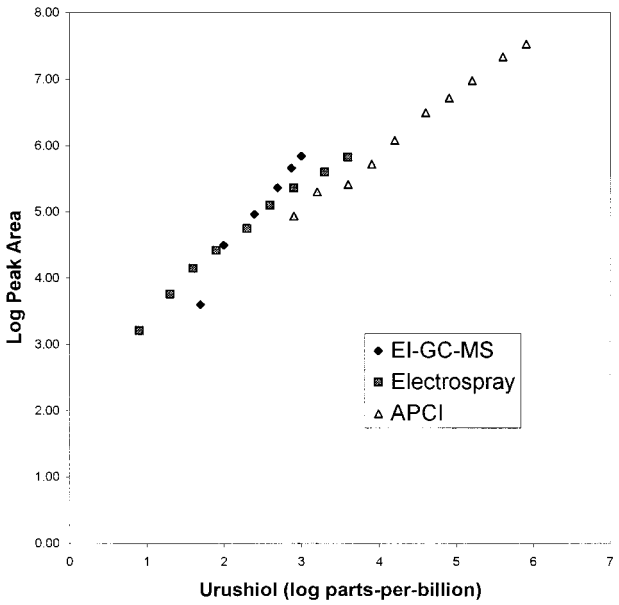

Figure 7. Multipoint calibration curves for negative ion APCI and ESI MS-MS and EI GC-MS determination of the C_{17} triene urushiol congener.

fragmentation yielded a series of predictable daughter ions with increased specificity. APCI-LC-MS-MS calibration conformed well to a quadratic response curve over a three-decade concentration range—negative ion ESI detection limits were in the low picograms per microliter range, ~100-fold lower than those of APCI.

The tentative identification of new urushiols from a California Western poison oak specimen, C_{17} tetraene and C_{15} saturated, demonstrates the potential for application of API-MS in natural products studies, pharmacognosy, and related areas of research. Further investigation is needed to develop improved HPLC separations for the extremely lipophilic urushiols.

ACKNOWLEDGMENT

We thank Professor William Epstein, M.D. (University of California, San Francisco School of Medicine Department of Dermatology), for generously providing an authentic urushiol sample and for helpful suggestions regarding the biological activity of poison oak. James Cone, M.D., of the CDHS Division of Occupational Health provided technical support and guidance and instigated the laboratory's work with poison oak allergens.

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Received for review October 12, 2001. Revised manuscript received January 18, 2002. Accepted January 18, 2002.

JF011364T