

**Table II. Drift Times and Calculated Reduced Mobilities ( $K_0$ ) for Negative Ions at Two Temperatures**

Temperature, °C	Drift time, milliseconds	$K_0$ , <sup>a</sup> cm <sup>2</sup> /V sec
125	4.90	3.33
	5.43	3.01
	5.87	2.78
	6.18	2.64
190	4.18	3.39
	4.66	3.02
	5.16	2.75
	5.51	2.57

<sup>a</sup>  $K_0 = \frac{1}{\tau} \cdot \frac{l}{E} \cdot \frac{273}{T} \cdot \frac{p}{760}$  where  $\tau$  = drift time,  $l$  = length of cell (6 cm),  $E$  = field gradient,  $T$  = absolute temperature, and  $p$  = pressure.

and only affects the relative concentration of ionic species slightly by shifting the equilibrium to favor the higher water clusters.

When air is present in the carrier gas, and the negative species are a mixture of electrons and water-clustered negative ions (Figure 5), the addition of water does result in a decrease of electrons with the formation of larger amounts of the negative ionic species. These observations tend to suggest that removal of water from the carrier gas of an EC detector is more important when air and other electron-capturing contaminants are present.

While a more complete and definitive work is needed to determine the exact effect these observations would have on the EC detector in its pulsed mode of operation with an argon/methane mixture, some data have been obtained. The addition of a 10-ml sample of argon/10% methane to the nitrogen carrier gas results in some formation of methane ion-molecules

of lower mobilities by reaction with the water-clustered protons which remain the dominant species. In the negative mode, the presence of the argon/methane mixture gives about the same results as does water; in the presence of air, the relative electron concentration decreases with formation of greater amounts of ion-molecules; when only electrons are present as in pure nitrogen, the argon/methane has little effect on the electron level. These effects are all a function of temperature. Work is currently under way to more completely define the pulsed mode of operation.

## CONCLUSIONS

These data indicate that the presence of small amounts of oxygen or similar electron-capture compounds in a carrier gas for an EC detector will considerably reduce sensitivity and linearity by formation of reactive negative ion-molecule complexes from the electrons. This effect is greater at lower temperatures and is somewhat related to water concentration. These, and other similar studies, provide a means of understanding many aspects of the EC detector mechanism and characteristics. Recent work (6) has produced evidence for both the direct and intermediate ion types of dissociative capture with halogen compounds. Other work is in progress with the plasma chromatograph to identify the reactions occurring with electron capture and to elucidate the kinetics of these processes.

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# Identification of Noncannabinoid Phenols in Marijuana Smoke Condensate Using Chemical Ionization Mass Spectrometry

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INCREASING USE OF MARIHUANA has generated interest in the constituents of the plant. The cannabinoids have been studied extensively because certain of these phenolic compounds are psychotomimetically active (1). Since the principal way by which marihuana constituents are taken into the human body is by smoking, the composition of the smoke is of particular interest. The cannabinoids are efficiently volatilized and absorbed in the lungs during the smoking process (2), but little is known of the composition of the smoke, particularly in regard to other potentially physiologi-

cally active compounds. One class of compounds of concern is the noncannabinoid phenols.

Among the various noncannabinoid phenols that have been identified in the marihuana plant are eugenol and guaiacol (3) as well as ferulic acid and *p*-hydroxycinnamic acid (melilotic acid) (4). However, the noncannabinoid phenolic constituents of marihuana smoke have not been investigated. We report here the identification of various noncannabinoid phenols in marihuana smoke condensate using gas chromatography/mass spectrometry (GC/MS) and the chemical ionization (CI) mass spectra thus generated.

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## EXPERIMENTAL

**Procedure.** Cigarettes prepared from female Mexican marihuana were smoked by a negative-pressure smoking machine. The smoke was condensed in a series of ice-cooled traps. The condensate was then washed from the traps with a 1:1 mixture of methylene chloride and ethanol and the solvent and water were removed under reduced pressure. The residue was taken up in methylene chloride and filtered. From 100 cigarettes was obtained 4.6 grams of soluble material.

The methylene chloride solution was extracted with a saturated aqueous sodium bicarbonate solution and then with 0.1*N* aqueous sodium hydroxide solution. Some phenols were contained in the sodium bicarbonate extract and others in the sodium hydroxide extract. The aqueous solutions were acidified and extracted with ether. The ether solutions were dried, filtered, and the solvent was evaporated. The material extracted by sodium bicarbonate accounted for 3% (138 mg) of the weight of the methylene chloride soluble condensate, and the material extracted by sodium hydroxide accounted for 8% (368 mg).

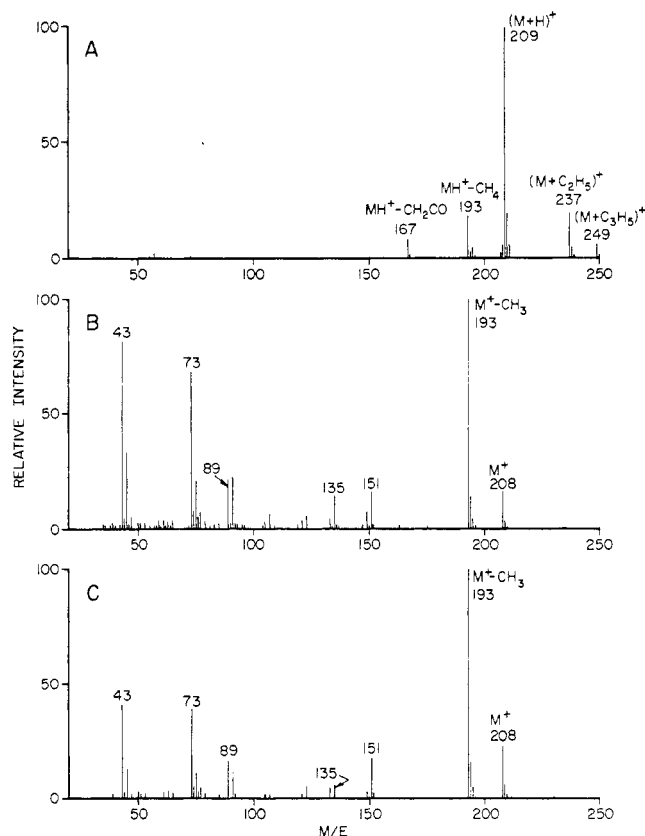
The solvent was evaporated and each residue was silylated using *N,O*-bis(trimethylsilyl)trifluoroacetamide. The derivatized material was then examined by GC using a 3-m stainless steel column packed with 1% OV-17 on 100/120 mesh Gas Chrom Q and flame-ionization detection. The column temperature was programmed from 75 to 300 °C at 6°/min.

**Instrumentation.** After examination of the derivatized extracts by conventional GC, the column was placed in a Varian Aerograph Model 1740 gas chromatograph connected directly to a Finnigan Model 1015 quadrupole mass spectrometer equipped with a chemical ionization source. No separator or splitter was used. The flow of carrier gas was adjusted to give ion source pressures which were previously found to permit maximum mass spectral sensitivity. For methane, this pressure was approximately 0.4 Torr, and for helium, approximately 0.3 Torr. The pressure measurements were made using a McLeod gauge connected to the inside of the ion block by a metal tube inserted through the probe inlet. The resulting flow rates corresponded to 30 ml/min for helium and 24 ml/min for methane as measured by a mass flow meter inserted in the carrier gas inlet line. The ion source (5) is differentially pumped by a 1200 l./sec diffusion pump. The ion source temperature was maintained at 200 °C and the ionization voltage at 100 eV.

The mass spectrometer was coupled to a Systems Industries 250 data system which provides both scan control of the mass spectrometer and processing of the mass spectral data.

## RESULTS AND DISCUSSION

Chemical ionization (CI) mass spectrometry has been shown to complement electron impact (EI) mass spectrometry effectively in the structural analysis of a wide variety of organic compounds (6-8). Methane and isobutane have been the reactant gases most commonly used in CI mass spectrometry. The CI mass spectra obtained using these reactant gases characteristically show abundant protonated molecule ions, many times even when the corresponding EI mass spectra show no detectable molecular ions. However, for providing a "fingerprint" identification of a compound, the EI mass spectrum is preferred because it generally shows a more detailed fragmentation pattern.



**Figure 1.** Mass spectra of the TMS derivative of *p*-hydroxyacetophenone

- A. Methane CI-MS
- B. High-pressure helium MS
- C. EI-MS

Another notable feature of CI mass spectrometry is that it permits the coupling of a gas chromatograph directly to the mass spectrometer without use of a separator or splitter (9, 10). In this mode of operation, the GC carrier gas also serves as the reactant gas in the mass spectrometer ion source. When methane is used as the carrier/reactant gas, typical CI mass spectra are obtained. Furthermore, the mass spectra obtained using helium as the carrier/reactant gas are very similar to conventional EI mass spectra (10). Therefore, it is possible to obtain either CI or EI-like mass spectra merely by valving in the appropriate carrier/reactant gas (11).

One of the compounds identified in this study as a component of marihuana smoke was *p*-hydroxyacetophenone. Figure 1 shows the mass spectrum of the trimethylsilyl derivative of *p*-hydroxyacetophenone determined in three ways. Spectrum A is the normal CI spectrum using methane as carrier/reactant gas; Spectrum B is an EI-like spectrum using helium as carrier/reactant gas; and Spectrum C is a conventional EI spectrum obtained by introducing a pure sample of the TMS derivative into the mass spectrometer by means of the direct probe. These examples demonstrate the simplicity of the methane CI mass spectrum in contrast to the EI and the high-pressure helium mass spectra. The similarity between the

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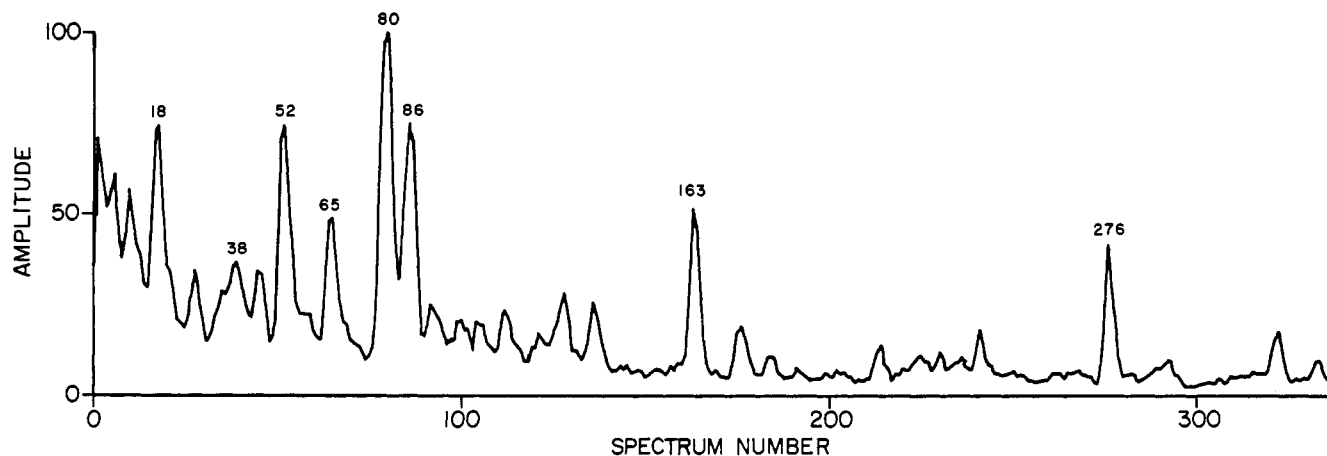


Figure 2. Computer reconstructed gas chromatogram of the TMS derivatives from the sodium bicarbonate extract of female Mexican marihuana smoke condensate

Table I. Composition of the Sodium Bicarbonate Extract

Compound identified as TMS derivative	Spectrum number (Figure 2)	Extract, %
Hexanoic acid	18	5.7
Heptanoic acid	38	3.8
Furoic acid	52	3.1
Octanoic acid	65	4.0
Benzoic acid	80	9.3
Catechol	86	12.1
<i>p</i> -Hydroxyacetophenone	163	2.6

last two spectra is evident. Recently, Einolf and Munson (12) reported that for most of the compounds they studied, the mass spectra obtained using helium as a reactant gas show virtually complete dissociative charge exchange, and that the very small amounts of molecular ions observed are apparently produced by direct electron impact and not by charge exchange reactions of  $\text{He}^+$ . In our work, we have not attempted to determine the mechanisms by which ionization of sample molecules occurs at high pressures of helium. However, it has been our experience that all of the prominent peaks in EI mass spectra will also be the prominent peaks in the corresponding high-pressure helium mass spectra. In a preliminary study, we have found that a computer program similar to that described by Hertz, Hites, and Biemann (13) can be used successfully to identify high-pressure helium mass spectra of drugs using a reference library of EI mass spectra. The relative intensities of the molecular ions are generally lower in the high-pressure helium mass spectra, and small differences are observed in the relative intensities of fragment ions. It also should be noted that significant protonated molecule ion peaks are often observed in both EI and high-pressure helium mass spectra obtained using a chemical ionization source (11).

Figure 2 is a computer-reconstructed chromatogram of the trimethylsilyl (TMS) derivatives of the sodium bicarbonate extracted material. The amplitude is the total ion-current response of the spectrometer and on the abscissa are spectra recorded during the run. A mass spectrum for a particular peak is obtained by recalling from the computer the spectrum

corresponding to the peak maximum. This chromatogram was obtained using helium as carrier gas. A similar run was made using methane as carrier and reactant gas. After tentative identification on the basis of the mass spectra, authentic materials were obtained, converted to their TMS derivatives, and both types of spectra were determined using helium and methane as carrier gas. A comparison of the two types of spectra obtained from the authentic materials was made with the corresponding spectra from the extract. In addition, it was established that the corresponding GC retention times were identical. Table I lists the components of the sodium bicarbonate extract which were identified on the basis of the congruence of spectral and GC data. Two phenols and several simple acids were identified in this fraction. The relative amount of each component has been estimated on the basis of a comparison of peak area to the entire area under the chromatogram obtained on a conventional GC. These data are not strictly quantitative and are meant to serve only as an indication of the relative amounts of the various materials in the smoke condensate. The approximate percentage of a compound in the total smoke can be determined by multiplying the extract per cent by 0.138/4.6.

The acids identified in this study have all been identified in tobacco and tobacco smoke but to our knowledge these acids have not been previously reported as constituents of marihuana smoke. We do not find the presence of these acids surprising or particularly significant. Because of the possible physiological effects of phenols, we think the presence of these compounds is more important.

Catechol has been identified in cigarette smoke (14), but has not been previously reported in marihuana smoke. *p*-Hydroxyacetophenone has the common name piceol and is present as the *D*-glucoside, called picein, in the needles and sprouts of *Pinus picea*, in willow barks such as *Salix discolor*, and in the English mistletoe, *Anelancher vulgaris* (15). *p*-Hydroxyacetophenone is also present in tobacco smoke, along with *o*- and *m*-hydroxyacetophenone (14). We were unable to detect any *o*- or *m*-hydroxyacetophenone in marihuana smoke.

As might be expected, considerably more phenolic materials were extracted by aqueous sodium hydroxide. Figure 3

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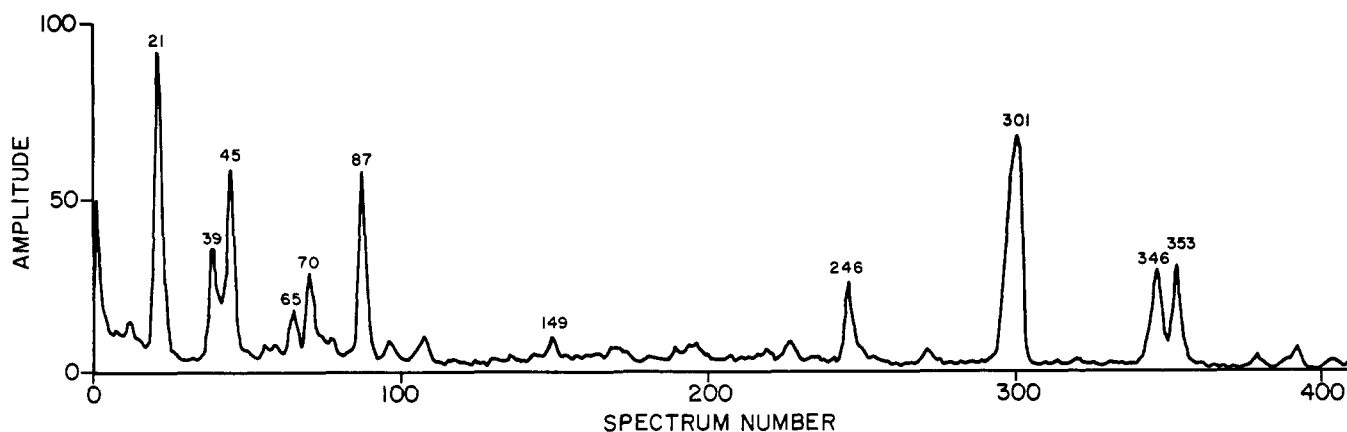


Figure 3. Computer reconstructed gas chromatogram of the TMS derivatives from the sodium hydroxide extract of female Mexican marihuana smoke condensate

is a reconstructed chromatogram of the TMS derivatives of the sodium hydroxide extracted material, and Table II lists the components of the extract which were conclusively identified. As for Table I, the approximate percentage of a compound in the total smoke can be determined by multiplying the extract per cent by 0.368/4.6.

In addition to *o*- and *p*-cresol which were identified, we also detected what we believe to be *m*-cresol. This material has a retention time between *o*- and *p*-cresol and corresponded exactly to that of *m*-cresol. However, we were unable to resolve this material in our GC/MS system and thus were unable to obtain a confirmatory mass spectrum on this minor component. Phenol as well as the three cresols has previously been identified in tobacco smoke (14). *p*-Ethylphenol has been positively identified. In addition, Spectrum 65 in Figure 3 is consistent with that of xylenol. As 2,4- and 3,5-xylenol have the same retention time in our GC system, we were unable to determine which isomer was present. The other four xylenol isomers were resolved and it was established that they were not present in this extract. Several xylenols as well as *p*-ethylphenol have been identified in tobacco smoke (14).

*p*-Vinylphenol has not been previously identified as a component of marihuana or tobacco smoke. Phenolic styrenes with a hydroxy function in the para position are particularly reactive, and until recently none of these derivatives has been identified in either marihuana or tobacco smoke. It appears that the formation of the TMS derivative of this phenol in stabilizing it with respect to reactivity and polymerization thus allows time for chromatography and analysis. This technique was used by Leach and coworkers in the identification of 4-vinylcatechol in cigarette smoke (16).

After tentatively identifying *p*-vinylphenol from the mass spectra, we prepared a sample by thermally decarboxylating *p*-hydroxycinnamic acid after the method of Schmid and Karrer (17). We immediately silylated the product and used this material as our standard. The high-pressure helium and methane CI mass spectra of the synthetic material were identical to the spectra obtained from the corresponding component of the smoke condensate.

It seems reasonable that *p*-vinylphenol results from thermal decarboxylation during the smoking process of the melilotic

Table II. Composition of the Sodium Hydroxide Extract

Compound identified as TMS derivative	Spectrum number (Figure 3)	Extract, %
Phenol	21	7.6
<i>o</i> -Cresol	39	9.2
<i>p</i> -Cresol	45	
<i>p</i> -Ethylphenol	70	
<i>p</i> -Vinylphenol	87	2.1
4-Hydroxy-3-methoxystyrene <sup>a</sup>	149	0.5
Myristic acid	246	4.6
Palmitic acid	301	35.2
Stearic acid	346	10.8
Linolenic acid	353	4.9

<sup>a</sup> Tentative identification, see text.

acid which is reported to be present in the marihuana plant (4). If so, it might be expected that 4-hydroxy-3-methoxystyrene might also be present, since this compound would result from the decarboxylation of ferulic acid which is known to be present in marihuana (4). In fact, the most abundant ion in the methane CI mass spectrum of the GC peak corresponding to Spectrum 149 of Figure 3 occurs at *m/e* 223 and is a protonated molecule ion, a conclusion supported by the presence of adduct ions at *m/e* 251 and 263. This is consistent with the TMS derivative of 4-hydroxy-3-methoxystyrene, although its identity has not been confirmed by comparison with an authentic sample.

Since melilotic acid and ferulic acid are also present in tobacco, we suggest that these phenolic styrenes in addition to 4-vinylcatechol might also be present in tobacco smoke and perhaps could be identified using these techniques.

One report emphasizes the physiological effects of phenols in smoke; included are cocarcinogenesis and ciliostasis (18). For this reason, we think it important that the composition of marihuana smoke be determined and that the physiological effects of the constituents be studied and compared to those of tobacco smoke.

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