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ANALYSIS OF THE ACIDIC FRACTION OF MARIJUANA SMOKE CONDENSATE BY CAPILLARY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

A method for the analysis of organic acids and phenols isolated from marijuana smoke condensate has been developed. Comparative analyses of standard tobacco, Mexican, and Turkish marijuana smoke condensates carried out by means of capillary gas chromatography indicated both qualitative and quantitative changes in the constituents of chromatographic profiles. Samples were converted to volatile derivatives by methylation and trimethylsilylation; whereupon, 49 aliphatic acids, aromatic acids, and phenolic compounds were identified by means of capillary gas chromatography–mass spectrometry.

INTRODUCTION

It is widely suspected that many fundamental problems associated with limited understanding of the pharmacological and toxicological effects of marijuana may have their origin in the insufficient chemical characterization. Although much progress has been made in the recent years[1], marijuana smoke still remains a poorly defined conglomerate of chemicals. Whereas much attention has been given to the many effects of a major marijuana constituent, Δ^9-tetrahydrocannabinol, this substance and marijuana are not always pharmacologically and toxicologically synonymous. Thus, activity can sometimes be located in fractions of the plant extract that contain no Δ^9-tetrahydrocannabinol or other related substances (*e.g.*, refs. 2 and 3).

Detailed knowledge of the composition of marijuana smoke will be urgently needed for future studies of its physiological effects. This paper is an extension of the characterization of marijuana plant and smoke materials performed in this laboratory[4–6].

Smoke fractionation is accomplished by a modified procedure of Schmeltz *et al.*[7] that has been used for isolation of polynuclear aromatic hydrocarbons from

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marijuana and tobacco smoke[6]. The acidic fraction yielded as a result of the fractionation scheme has now been chemically characterized in this study. Phenolic substances and aromatic and aliphatic acids that can be volatilized for gas chromatographic (GC) investigations through derivatization have been of primary interest.

General interest in acidic components stems from the fact that both phenols and acids contained in tobacco smoke were found to be a contributory factor in tumor formation and to possess a cilia-inhibiting activity[8,9]. Whereas positive identification of all constituents of marijuana smoke acidic fraction, biological testing and physiological interpretation of such studies remain a long-term goal, a method for selective screening for unusual compounds is of utmost importance in the beginning stages of such investigations. One approach that can facilitate orientation in any complex fraction of marijuana smoke is using tobacco smoke as a "baseline material" for comparison and screening the available samples for obvious qualitative and quantitative differences.

The methodology for analysis of the acidic fraction of marijuana smoke described in this paper consists of solvent partitioning, purification and selective fraction enrichment by gel chromatography, followed by conversion of sample components to volatile derivatives for GC. Efficient glass capillary columns further provide a degree of resolution required for the mass spectrometric (MS) investigation of the sample components. High-resolution GC profiles of the fractions obtained from Mexican and Turkish marijuana and standard tobacco smoke condensates have been compared. Also, numerous components of the acidic fraction from Mexican marijuana have been identified in this work.

EXPERIMENTAL AND RESULTS

Sample preparation

One hundred cigarettes each of Mexican and Turkish marijuana (National Institute of Drug Abuse, Rockville, Md., U.S.A.) and standard tobacco (Tobacco–Health Research Institute, University of Kentucky, Lexington, Ky., U.S.A.) prepared from equal weights, were smoked with a standard smoking machine[10] at approximately 35-ml puff volume for a duration of 2 sec at a frequency of 1 min. The content of Δ^9-tetrahydrocannabinol was 2.8%, for Mexican, and 0.3% for Turkish marijuana, respectively. The smoke condensates were separately collected in acetone-filled traps cooled with dry ice–acetone.

Some of the acetone was removed *in vacuo* at 40° and the brown oily residue redissolved in 250 ml of methylene chloride. The extract was partitioned three times with 250 ml of 1 *N* sodium hydroxide solution, and the aqueous layer was washed twice with 300 ml of methylene chloride. The aqueous extract was subsequently adjusted with hydrochloric acid to pH 1.0, and the organics of acidic nature were re-extracted with two 450-ml volumes of methylene chloride. The extracts were dried over magnesium sulfate and filtered. The solvent was finally removed *in vacuo*.

The acidic residues from Mexican marijuana and standard tobacco were weighted, yielding 6.25 and 2.05 mg/cigarette, respectively. Whereas much of this material is obviously non-volatile, the primary objective of this study has been to recognize differences in the constituents of marijuana and tobacco smoke that are either volatile or can be volatilized through derivatization. Addition of an internal

standard to the total acid fractions revealed that they contain only about 20% of elutable material.

Selection of analytical column for high-resolution gas–liquid chromatography

It has been well established that both acid and phenolic compounds are more easily chromatographed in the gas phase when converted to less polar derivatives[11–14]. When acidic stationary phases are coated on the wall of a capillary column, highly volatile acids and phenols can also be chromatographed without derivatization. Such cases were demonstrated by Averill[15] and Zoccolillo *et al.*[16]. More recently, Hrivňák *et al.*[17] have also demonstrated an efficient separation of C_2–C_6 fatty acids with steel capillary columns coated with Ucon LB-550-X, with the addition of phosphoric acid.

It has been our experience in this work that adequate glass capillary columns can be prepared by coating the etched glass-surface[18] with both FFAP ("free fatty acid phase"; Supelco, Bellafonte, Pa., U.S.A.) and Trimer Acid (Applied Science Labs., State College, Pa., U.S.A.) with addition of phosphoric acid. Whereas such columns perform well when freshly prepared, the resolution of free fatty acids diminishes rapidly with time. This phenomenon is illustrated in Fig. 1. Because the long-term column stability is maintained for hydrocarbons, the loss of resolution for

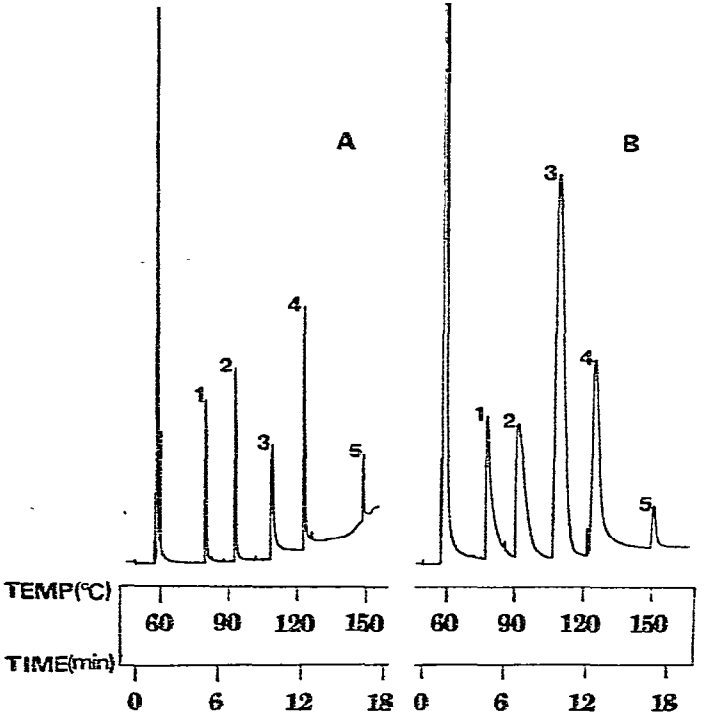

Fig. 1. Chromatograms of a standard mixture of free fatty acids. (A) Chromatogram obtained with a freshly prepared column and short conditioning at 180°. (B) Chromatogram obtained after 20 h conditioning at 180°. 1 = Propionic acid; 2 = butyric acid; 3 = valeric acid; 4 = hexanoic acid; 5 = octanoic acid. Conditions: 2ᶜ m × 0.25 mm I.D., glass capillary column coated with Trimer Acid + 30% phosphoric acid; injector temperature, 220°; detector temperature, 210°; splitting ratio, 1:50.

free fatty acids can be attributed to a loss of surface acidity and/or deactivation that is most likely due to an easy migration of alkali ions within the silica framework[19]. Perhaps, this problem could be overcome by a more permanent surface modification.

Owing to its good temperature stability and selective properties, FFAP was chosen as the stationary phase for separations of derivatized acids and phenols. The glass capillary column (20 m × 0.25 mm I.D.) was prepared by etching with dry hydrogen chloride[18,20] and was coated dynamically with a 15% solution of FFAP in methylene chloride by the mercury-plug method recommended by Schomburg *et al.*[21]. A Varian Model 1400 gas chromatograph with a modified splitting injector, linear temperature programmer and flame ionization detector was used for all GC separations.

Comparison of chromatographic profiles

In order to obtain volatile derivatives, a methylation technique reported by Middleditch and Desiderio[13] was applied to aliquots of the acidic fractions of marijuana and tobacco smoke condensates. Fifty to one hundred μl of the extract were evaporated to dryness in a micro-vial, and the residue was dissolved in 25 μl of the 0.2 *M* solution of trimethylanilinium hydroxide (Methelute; Pierce, Rockford, Ill., U.S.A.), and 2.5 μl of the solution were immediately injected in to the gas chromatograph (splitting ratio, 1:50).

The comparative analyses of the three investigated total acidic fractions after methylation are shown in Fig. 2, demonstrated both qualitative and quantitative differences in all fractions. Since MS data acquired on the resolved peaks showed that these chromatograms were primarily dominated by fatty acids, and that the derivatization of the total extract resulted in incomplete reaction of certain aromatic constituents, further selective fractionation by liquid column chromatography was found necessary. Two mg of the dry total acidic fraction dissolved in 0.5 ml of methanol were placed on a 25 × 0.8 cm I.D. DEAE-Sephadex A-25 (Cl^-) (weak anion exchanger) column and eluted with methanol as the mobile phase, taking advantage of the well-known phenomenon of reversible adsorption of aromatics by Sephadex gels. Whereas most non-volatile material was unretained and aliphatic acids were eluted in one column volume, phenolic substances and aromatic acids required a double amount of methanol for elution.

The fraction from all materials containing phenols and aromatic acids were evaporated to dryness and their equal aliquots methylated and analyzed on the same capillary column. Fig. 3 compares all chromatographic profiles of this aromatic fraction.

Gas chromatography–mass spectrometry

The FFAP glass capillary column was connected to the ion source of a Hewlett-Packard Model 5980A dodecapole mass spectrometer through an all-glass jet molecule separator (Scientific Glass Engineering). Electron-impact ionization spectra were obtained with an electron energy of 20 or 70 eV. Chromatographic peaks were scanned at the rate of 100 a.m.u./sec and mass spectra were recorded on oscillograph paper.

Many major acidic components of Mexican marijuana smoke were tentatively identified from mass spectra of their methylated derivatives. Since the methylation

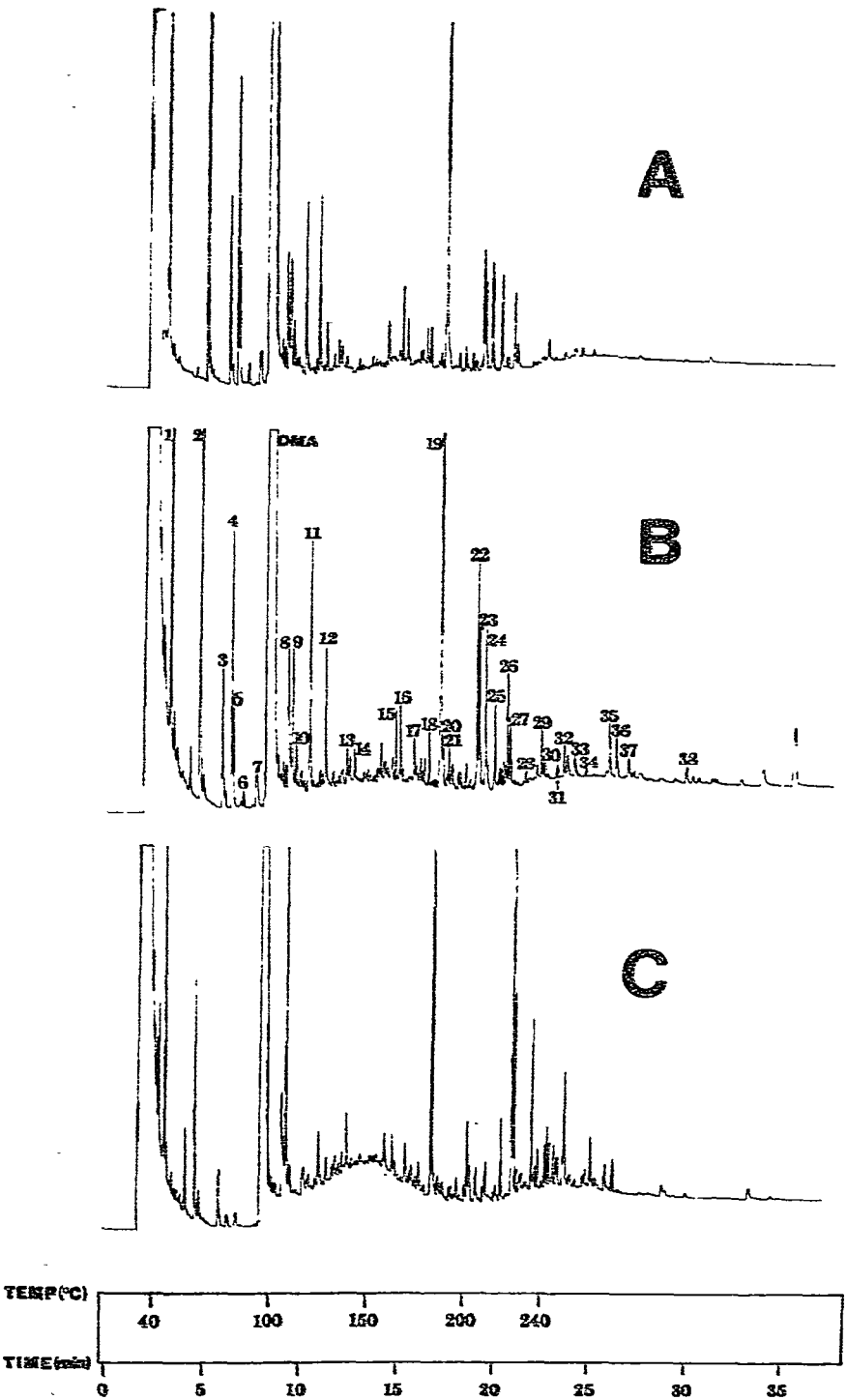

Fig. 2. Chromatograms of methylated total acidic extracts of cigarette smoke condensates. (A) Standard tobacco; (B) Mexican marijuana; and (C) Turkish marijuana. Conditions: 20 m × 0.25 mm I.D., glass capillary column coated with FFAP; injector temperature, 260°; detector temperature, 210°. Injected amount was approximately 60 μg of extract; splitting ratio, 1:50. For peak identification, see Table I; DMA = dimethylaniline.

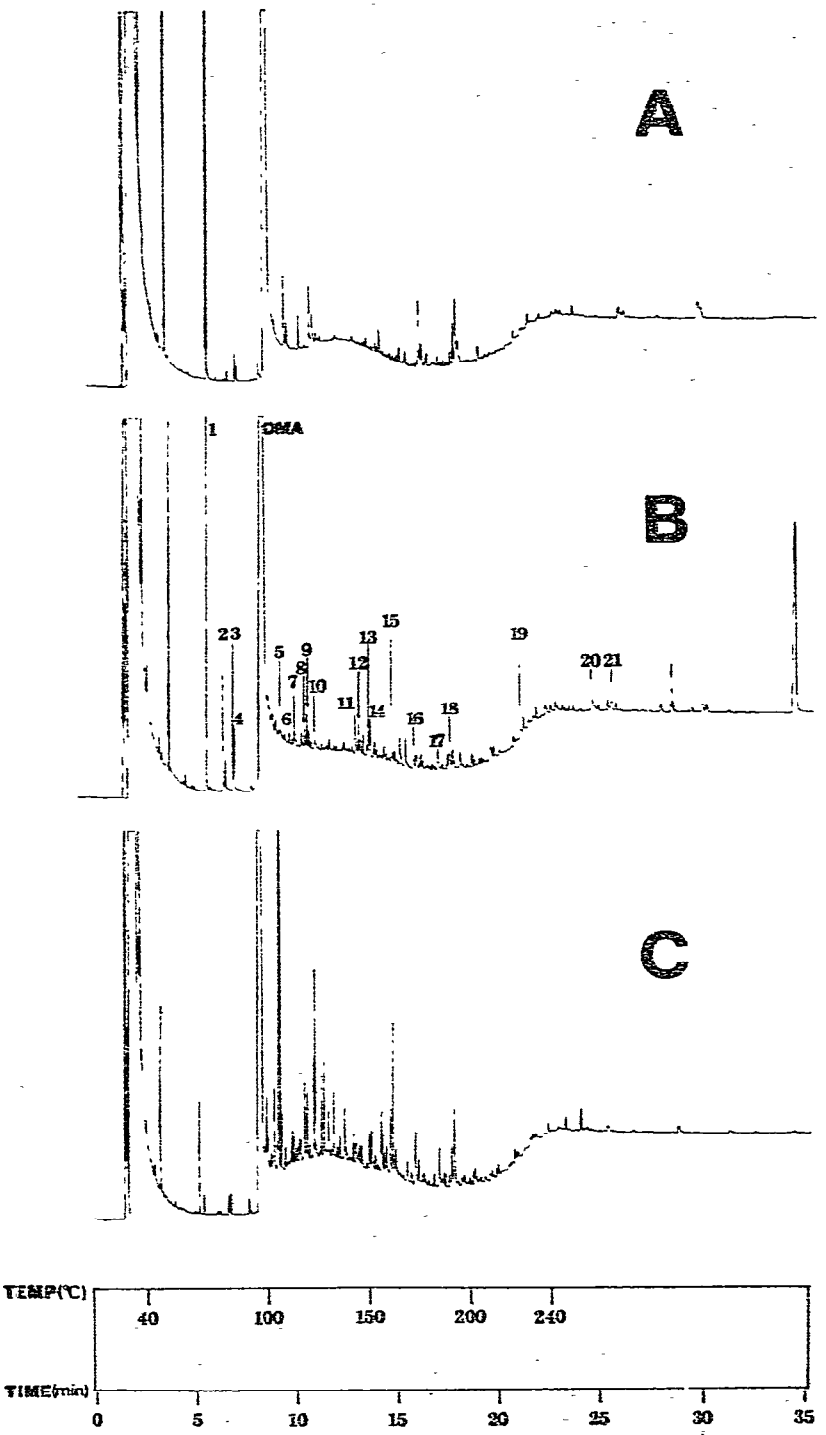

Fig. 3. Chromatograms of the methylated aromatic fraction obtained through DEAE-Sephadex liquid chromatography. (A) Standard Tobacco; (B) Mexican marijuana; and (C) Turkish marijuana. Conditions: same as in Fig. 2. For peak identification, see Table I.

procedure masks the possible presence of naturally occurring methoxy compounds within the aromatic fraction, silylation with N-methyl-N-trimethylsilyltrifluoroacetamide (Pierce), was carried out at 80° for 2 h to provide additional information. Chromatogram of the silylated fraction of Mexican marijuana aromatics is shown in Fig. 4.

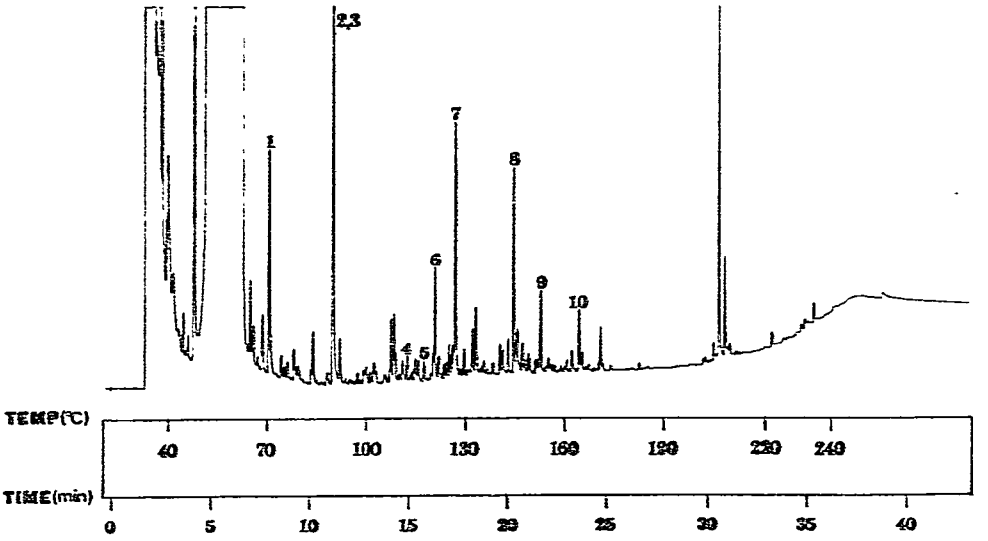

Fig. 4. Chromatogram of a silylated aromatic fraction (after liquid chromatography). Conditions: same as in Fig. 2. For peak identification, see Table I.

The results of the MS identifications of components of the total acidic fraction (Fig. 2) and methylated and silylated aromatic fractions (Figs. 3 and 4) are listed in Table I. In total, 49 components have been identified.

DISCUSSION

Whereas a limited number of acidic components have been reported in cannabis plant material[1] and also, more recently, in marijuana smoke condensate[22], this study illustrates for the first time the real complexity of such mixtures and provides an efficient methodology for identification of the elutable acidic components. It also points out that much further work will be necessary to identify the many trace components, some of which might bear physiological significance.

A significantly higher total acidic content of marijuana smoke compared with tobacco smoke could be important in view of the possible implication of such substances in co-carcinogenic and ciliostatic activities[8,9]. A role of various acidic compounds in this direction must first be substantiated by detailed analytical data on both volatile and heavy constituents and, most importantly, biological experiments.

The comparisons of both the total and the aromatic fractions of acidic constituents, as shown in Figs. 2 and 3, indicate a higher complexity and total amounts for marijuana as compared with tobacco. Also, both qualitative and quantitative dif-

TABLE I

COMPOUNDS IDENTIFIED IN ACIDIC FRACTION OF MEXICAN MARIJUANA

Compound	*Peak Number*		
	Fig. 2	*Fig. 3*	*Fig. 4*
Hexanoic acid	1	—	—
Phenol*,**	2	1	—
o-Cresol*	3	2	—
p-Cresol*	4	3	—
m-Cresol*	5	4	—
Furoic acid*	6	—	—
Nonanoic acid	7	—	—
Decanoic acid	8	—	—
Benzoic acid*	9	5	—
o,p-Divinylphenol	10	6	2, 3
Catechol*	—	7	1
Glutaric acid	11	—	—
Phenylacetic acid*	—	8	—
o-Isopropenylphenol	—	9	6
m-Hydroxy-*p*-methoxystyrene	12	10	1
Dodecanoic acid	13	—	—
2,4-Dihydroxy anisole	14	11	8
o-Hydroxybenzaldehyde*	—	12	4
Phenylpropionic acid	—	13	—
Phenylisopropionic acid	—	14	—
o-Hydroxyacetophenone	15	15	5
Tetradecanoic acid*	16	—	—
Olivetol	17	16	10
3-Isopropyl-5-hydroxybenzaldehyde	—	17	—
2,4-Dihydroxybenzaldehyde	18	18	9
p-Hydroxybenzyl 2-butenyl ketone	—	19	—
Palmitic acid*	19	—	—
Palmitoleic acid*	20	—	—
Palmitolenic acid	21	—	—
Stearic acid*	22	—	—
Oleic acid*	23	—	—
Linoleic acid*	24	—	—
Linolenic acid*	25	—	—
Arachidic acid*	26	—	—
Eicosenoic acid	27	—	—
Eicosadienoic acid	28	—	—
Behenic acid	29	—	—
Erucic acid	30	—	—
Tricosanoic acid	31	—	—
2-Ethyl-3-hydroxy-5-pentylbenzoic acid	32	20	—
2-Vinyl-3-hydroxy-5-pentylbenzoic acid	33	21	—
Lignoceric acid	34	—	—
Tetracosatetraenoic acid	35	—	—
Hexacosanoic acid	36	—	—
Hexacosadienoic acid	37	—	—
Octacosanoic acid	38	—	—
Also identified under the solvent peak:			
2-Methylbutanoic acid			
3-Methylbutanoic acid			
4-Pentenoic acid			

* Denotes compounds compared with standards.

** Since anisol was recently found[26] to originate from the thermal decomposition of trimethylanilinium hydroxide, phenol cannot be quantitated from the present chromatograms.

ferences between Mexican and Turkish marijuana profiles are noteworthy. Although probably without any physiological meaning, it is interesting to observe that the ratio of fatty acids in Turkish marijuana is strikingly different from Mexican marijuana and standard tobacco, which are of somewhat similar composition.

Whereas the complete identification of all mixture components was not within the scope of this study owing to a lack of both standard compounds and additional structural information, many potentially interesting compounds were found in the smoke condensate of Mexican marijuana. Just as with the hypothesis that many simple phenolic compounds present in tobacco smoke condensate have their origin in the carbohydrate content of tobacco leaf[23], the same is likely to be true for marijuana. However, it can be easily speculated that certain cannabinoids could give rise to various substituted phenols upon pyrolysis. In fact, recent studies of Küppers *et al.*[24] on the model pyrolysis of cannabidiol appear to substantiate this idea.

Evidence presented by Burstein *et al.*[2,25] that certain substituted phenols present in the extracts of *Cannabis sativa* are strong inhibitors of prostaglandin biosynthesis indicates that some of the phenolic constituents found in this work may bear pharmacological significance. We have found one of the substances with proven physiological activity[25], olivetol, to be present in the acidic fraction of Mexican marijuana smoke in appreciable quantity.

Finally, it should be pointed out that, whereas the present methodology has been developed specifically for fractionation, analytical separation, and identification of the elutable acidic components of marijuana smoke condensate, its wider applicability to any analysis of acidic compounds present in mixtures of comparable complexity is likely.

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