components in a mixture is contained in the complete spectra and (b) the ability to maximize the sensitivity for all components by obtaining chromatographic data at the optimum wavelengths from a 3-D chromatogram. An implication of the second advantage is that the selection of optical conditions can compensate for poor chromatographic resolution.

ACKNOWLEDGMENT

The authors gratefully acknowledge B. J. Norris for recording spectra of the nucleotide bases on a Cary 14.

LITERATURE CITED

(1) L. R. Snyder and J. J. Kirkland, "Introduction to Modern Liquid Chromatography", J. Wiley and Sons, New York, N.Y., 1974, p 142ff.
(2) G. Zweig and J. Sherma, *Anal. Chem.*, **46**, 73R (1974).
(3) C. D. Carr, *Anal. Chem.*, **46**, 743 (1974).
(4) R. E. Santini, M. J. Milano, and H. L. Pardue, *Anal. Chem.*, **45**, 915A (1973).
(5) M. J. Milano, H. L. Pardue, T. E. Cook, R. E. Santini, D. W. Margerum, and J. M. T. Raycheba, *Anal. Chem.*, **46**, 374 (1974).
(6) M. J. Milano and H. L. Pardue, *Anal. Chem.*, **47**, 25 (1975).
(7) R. M. Wightman, R. L. Scott, C. N. Reilley, R. W. Murray, and J. N. Burnett, *Anal. Chem.*, **46**, 1492 (1974).
(8) J. W. Strojek, G. A. Gruver, and T. Kuwana, *Anal. Chem.*, **41**, 481 (1969).
(9) E. E. Wells, Jr., *Anal. Chem.*, **45**, 2022 (1973).
(10) H. B. Mark, Jr., R. M. Wilson, T. L. Miller, T. V. Atkinson, H. Wood, and A. M. Yacynych, "The On-Line Computer in New Problems in Spectroscopy: Applications to Rapid Scanning Spectroelectrochemical Experiments and Time Resolved Phosphorescence Studies" in "Information Chemistry; Computer Assisted Chemical Research Design", S. Fujiwara and H. B. Mark, Jr., Ed., University of Tokyo Press, Tokyo, Japan, 1975, pp 3–28.
(11) K. W. Busch and G. H. Morrison, *Anal. Chem.*, **45**, 712A (1973).
(12) K. W. Busch, N. G. Howell, and G. H. Morrison, *Anal. Chem.*, **46**, 575 (1974).
(13) K. W. Busch, N. G. Howell, and G. H. Morrison, *Anal. Chem.*, **46**, 2074 (1974).
(14) F. L. Fricke, O. Rose, Jr., and J. A. Caruso, *Anal. Chem.*, **47**, 2018 (1975).
(15) D. G. Mitchell, K. W. Jackson, and K. M. Aldous, *Anal. Chem.*, **45**, 1215A (1973).
(16) K. M. Aldous, D. G. Mitchell, and K. W. Jackson, *Anal. Chem.*, **47**, 1034 (1975).
(17) L. B. Rogers, *C&EN*, April 15, 1974, p 18.
(18) K. M. Aldous and J. S. Garden, "Abstracts of Papers", 26th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio, March 1975, Abstract No. 434.
(19) A. Bylina, D. Sybilska, Z. R. Grabowski, and J. Koszewski, *J. Chromatogr.*, **83**, 357 (1973).
(20) A. M. Yacynych, Ph.D. Thesis, University of Cincinnati, Cincinnati, Ohio, 1975.
(21) L. C. Hansen and T. W. Gilbert, *J. Chromatogr. Sci.*, **12**, 458 (1974).

RECEIVED for review July 21, 1975. Accepted October 2, 1975. The authors gratefully acknowledge the financial support provided by National Science Foundation Grants GP-41981X for the RSS and GP-35979 for departmental computer equipment.

Analysis of Marijuana Samples from Different Origins by High-Resolution Gas–Liquid Chromatography for Forensic Application

Milos Novotny,* M. L. Lee, Chow-Eng Low, and Alain Raymond[1]

Department of Chemistry, Indiana University, Bloomington, Ind. 47401

A highly specific procedure for "fingerprinting" marijuana samples has been developed. The method consists of extraction of a marijuana sample, a single partition step, and the use of a precolumn concentration technique prior to gas chromatography. The high resolving power of glass capillary columns is essential for developing complex chromatographic profiles that are unique for a given sample. Characteristic profiles of nonpolar marijuana constituents are shown for selected samples from different geographical origins. Considerably higher specificity of this profile method over the conventional measurement of the relative concentrations of major cannabinoids is demonstrated. Thirty-eight profile constituents have been identified by combined gas chromatography–mass spectrometry.

Chromatographic methods have been implicated in at least three forensic applications concerning abuse of marijuana: (a) determination of whether an unknown sample of plant material contains marijuana; (b) determination of whether cannabis samples confiscated at different locations originate from a common lot; and (c) tracing of illicit marijuana samples to their geographical origin.

Generally, the first case is the most straightforward one and does not require sophisticated separation methods. Even when marijuana is blended with non-cannabis plants used as adulterants, combination of a simple histological technique with thin-layer chromatography appears to be sufficient (*1*) for positive identification. Tracing of illicit marijuana samples to their origin has been a considerably more complicated task; the literature concerning this problem is indeed abundant with many attempts to correlate sample composition with its origin.

It has been well-established that the various types of cultivated and wild marijuana or hemp differ considerably in their respective cannabinoid content. Cannabis plants from different parts of the world may vary from those producing almost exclusively cannabidiol to those producing predominantly Δ^9-tetrahydrocannabinol. Consequently, several workers (*2–6*) used the measurement of the relative concentrations of cannabidiol, Δ^9-tetrahydrocannabinol, and cannabinol (so-called "main cannabinoids") with the objective in mind of determining from which country each sample originates. In the course of such studies, several problems have become apparent that seriously limit this analytical approach: 1) It has been determined that, at least for the first several generations, the content of major cannabinoids produced by the plant is dependent upon the inherited properties of the seed, and that the genotype appears to be far more important than the influence of immediate geographical location and climate. Consequently, it has been suggested (*3*) that if cannabis seeds are shipped from one country to another for illegal cultivation, there is little valid basis for attempts to correlate the cannabinoid content with the place of origin. 2) Phillips et al. (*7*) have further observed a cyclic variation of cannabidiol and Δ^9-tetrahydrocannabinol during the growing season of an Indiana variety. The variation in cannabidiol content ranged

[1] Present address, Intersmat Instruments, 5 and 7 Allee Jean de la Fontaine, Pavillons-sous-Bois 93320, France.

from 0 to almost 70 mg/g of dry plant material within the period of 1 month. The variation in Δ^9-tetrahydrocannabinol was not as great, but ranged from 0 to 15 mg/g during a different period. Another trend observed was that Δ^9-tetrahydrocannabinol content was usually low on the same day that cannabidiol was high, and vice versa. It is obvious that, with such seasonal variations, the evaluation task becomes even more complicated. 3) Although the effects of plant processing and storage have not been investigated in great detail, they cannot be overlooked. Chemical changes of some cannabinoids with time have been observed (*4, 8, 9*).

While there seems to be a general agreement on the inherited composition of major cannabinoids, stronger environmental effects on minor cannabinoids or noncannabinoid constituents of the plant have never been ruled out. In fact, Turner et al. (*10*) pointed out that the propyl homologues of major cannabinoids might be more indicative of some geographical differences, and Strömberg (*11*) and de Zeeuw et al. (*12*) suggest that other constituents should also be considered for analysis.

Insufficient resolution of the many compounds present in a marijuana extract has been a serious drawback in the hitherto used methods; spectral methods, thin-layer chromatography, and conventional gas chromatography may give information on only several more abundant compounds. In fact, even gas-chromatographic separations of cannabinoid constituents with packed columns have been less than satisfactory.

High-resolution (glass) capillary GLC techniques, developed in our laboratory primarily for biochemical analyses, have also been used in two applications of forensic interest (*13, 14*). The degree of resolution and the limit of detection are often critical parameters in many forensic applications which involve chromatographic "fingerprinting". The latter problem is conveniently overcome by use of a precolumn sampling procedure (*15*) which permits reproducible and quantitative enrichment of trace organics. Such a step is essential in the development of meaningful profile comparisons.

Preliminary analyses of the extracts of marijuana samples from different origins performed in our laboratory indicated their high complexity. Their respective chromatograms appeared always different in certain details. Consequently, we have now developed a highly reproducible procedure with the specific objective to fingerprint marijuana samples. This method consists of Soxhlet extraction of marijuana with cyclohexane, partition of the extract between cyclohexane and nitromethane, and subsequent capillary gas chromatography of the cyclohexane fraction. Chromatographic profiles obtained by this method contain over 70 constituents (including cannabinoids) of which 38 were identified by combined gas chromatography–mass spectrometry in this work.

Although extensive correlation studies concerning geographical, genetic, and other factors were outside the scope of this study, there is significant evidence that complex chromatographic profiles are highly diagnostic for a given sample. It is demonstrated here with selected examples that this method is useful in cases where the conventional methods fail. Simplicity and reproducibility of this chromatographic profile procedure make it attractive for both forensic applications and chemotaxonomical studies of cannabis plants. In addition, the high resolving power of glass capillary columns combined with mass-spectral information may provide quite uniquely pharmaceutically important structural information on both unconventional cannabinoids and other biosynthetically related plant constituents.

EXPERIMENTAL

Sample Preparation. One-half gram of each marijuana sample (obtained in different batches from the National Institute of Mental Health, Rockville, Md.) were extracted with 100 ml of cyclohexane in a Soxhlet apparatus for 3 hr and then washed with 100 ml of nitromethane in a separatory funnel. The cyclohexane fractions were further evaporated to dryness in vacuo and re-dissolved in 7.6 ml of methylene chloride, from which 4 μl aliquots were used for analysis. All reagents were spectroquality solvents that were further checked prior to use for organic impurities that would interfere with the analyses.

Gas-Chromatographic Profiles. Four-μl aliquots of the methylene chloride solution were transferred by means of a 10-μl syringe to a concentration precolumn containing 2 mg of a highly deactivated support (*15*). The solvent was flushed out of the precolumn with helium gas at room temperature for 5 min. The precolumn was transferred to the injection port (*15*) of a Varian 1400 gas chromatograph which was modified to accommodate the precolumn. Concentration of the sample into the first part of the analytical column (kept at room temperature) was accomplished by thermal stripping of organics from the precolumn held at 250 °C. Sample trapping time of 20 min was found sufficient for reproducible and quantitative sample transfer into the capillary column. The oven temperature was subsequently increased to 70 °C and programmed to 240 °C at 2 °C/min for recording the gas-chromatographic profiles.

The column used in this study was an 11 m × 0.26-mm i.d., glass capillary column coated with SE-52 methylphenylsilicone stationary phase.

Gas Chromatography–Mass Spectrometry. The glass capillary column was directly connected to the ion source of a Hewlett-Packard Model 5980 A combined gas chromatograph/dodecapole mass spectrometer. The gas chromatograph was again modified for the use of a precolumn. Electron-impact ionization spectra were obtained with an electron energy of 70 eV. Perfluorotributylamine (PFTBA) was used for adjustment of relative intensities of *m/e* ratios over the used mass range. Spectra from capillary-column fractions were recorded on oscillographic paper. Rapid scanning times necessary to record representative spectra from sharp capillary-column peaks (*16*) allowed only nominal-mass spectral resolution.

RESULTS AND DISCUSSION

Selection of extraction and partition solvents for obtaining representative profiles, that are essential for comparisons, was of fundamental importance in this study. Although other solvents (e.g., dichloromethane used in the previous development of the precolumn concentration technique (*15*)) were found suitable for more exhaustive extraction of the plant constituents and resulted in more complex chromatograms, the cyclohexane–nitromethane system was chosen for different reasons. First, a great majority of more polar plant constituents are not initially extracted into cyclohexane. Consequently, relatively nonpolar marijuana constituents can be more easily chromatographed without thermal decomposition or eventual need for sample derivatization, making it easier to maintain analytical reproducibility for comparative purposes. The greater percentage of cannabinoids are further extracted into the nitromethane fraction, leaving the cyclohexane fraction much less dominated by overwhelming concentrations of these compounds and more useful for development of profiles. This partition step is highly reproducible. Nitromethane extracts were also investigated, but found of little value for fingerprinting purposes.

While the repetitive runs of identical samples were found to be highly reproducible, both qualitative and quantitative differences were found with numerous marijuana samples of different origin. Figure 1 demonstrates that differences can be observed with samples from different plants. As expected, the Turkish variety (low Δ^9-tetrahydrocannabinol content) is significantly different from the Mexican marijuana (chromatogram A vs. B, respectively). However, certain obvious differences appear to exist in the profiles of Mexican marijuana grown under different geographical

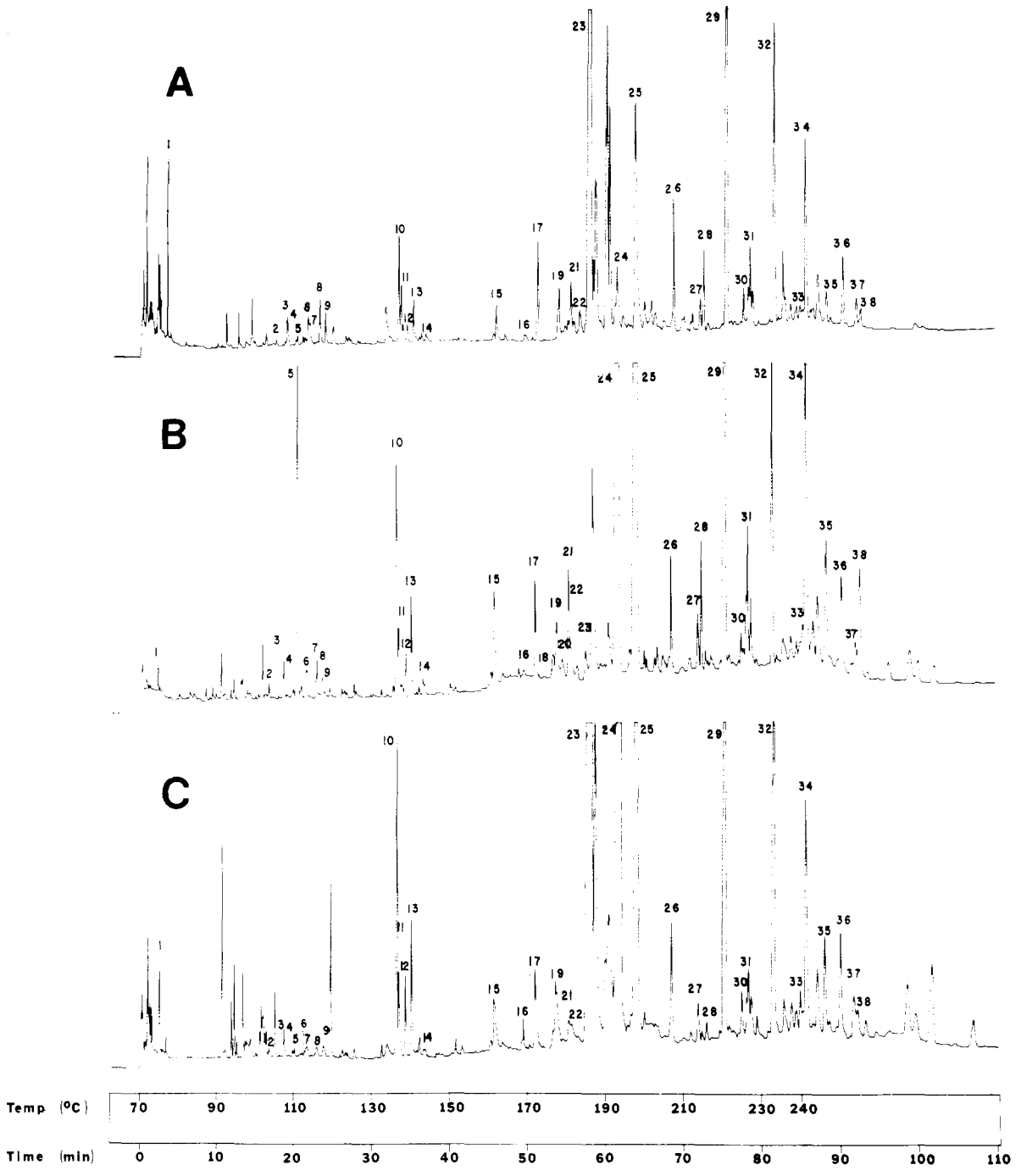

Figure 1. Capillary gas chromatograms of the cyclohexane fraction of three different marijuana samples

(A) Turkish marijuana, (B) Mexican marijuana, and (C) Indiana-grown Mexican marijuana. Column: 11 m × 0.26-mm i.d., glass capillary column coated with SE-52 methylphenylsilicone stationary phase. For peak identifications, see Table I

conditions (B vs. C, respectively) that could hardly be traced by conventional methods. Figure 2 shows subsequent analyses of two aliquots of the same material that were separately extracted, partitioned, and chromatographed, indicating good reproducibility.

The greater degree of resolution within the cannabinoid region (central part of the chromatograms) is a distinct advantage of glass capillary columns over conventional packed columns. Because of the considerably lower resolving power of the latter, it is very likely that the major cannabinoids quantitated in many previously reported communications as single peaks are indeed mixtures. This fact may, perhaps, be partly responsible for numerous discrepancies and controversies on correlations of marijuana composition with various factors that appear throughout the literature. Certain problems with limited chromatographic resolution of marijuana components were already pointed out (*12, 17*).

The method of comparing relative concentrations of the three major cannabinoids for tracing illicit samples to their origin, proposed originally by Davis et al. (*2*), is apparently used extensively in routine forensic work (*18*). We would like to point out, with the aid of Figures 3 and 4, that this approach can easily lead to wrong conclusions. Figure 3 compares chromatograms of the three major cannabinoids obtained from the extracts of Colombian (A) and Mexican

Table I. Analytical Data and Structure Identification

Peak No.	Mol wt	Formula	Significant *m/e* (rel. int.)	Compound
1	96	C_6H_8O	96(100), 67(54), 95(45), 81(25), 53(22)	2,4-Dimethylfuran
2	204	$C_{15}H_{24}$	69(100), 41(80), 135(42), 149(21), 163(20)	6-Methyl-2-(1-methyl-3-cyclohex-1-enyl)hepta-1,5-diene
3	202	$C_{15}H_{22}$	43(100), 134(70), 91(64), 69(63), 202(14)	3,8,10(15)-Cadinatriene
4	220	$C_{15}H_{24}O$	43(100), 161(70), 187(44), 202(21), 220(4)	12-Hydroxy-2,8-eudesmadiene
5	222	$C_{12}H_{14}O_4$	149(100), 177(22), 105(8), 93(4), 222(1)	Diethyl phthalate[a]
6	220	$C_{15}H_{24}O$	43(100), 91(55), 93(53), 136(38), 220(6)	2-Hydroxy-3,7-cadinadiene
7	220	$C_{15}H_{24}O$	136(100), 41(96), 79(75), 91(68), 220(18)	2-Hydroxy-3,7(11)-cadinadiene
8	202	$C_{15}H_{22}$	43(100), 91(74), 93(73), 131(37), 202(31)	2-(3-Methyl-2-butenyl)-*p*-mentha-2,6-diene
9	202	$C_{15}H_{22}$	91(100), 131(52), 159(23), 187(26), 202(8)	Eudesma-2,6,8-triene
10	250	$C_{15}H_{22}O_3$	43(100), 58(87), 95(39), 109(27), 249(8)	5(9-Ketodecyl)-2-furfuraldehyde
11	250	$C_{17}H_{30}O$	43(100), 58(87), 95(31), 137(7), 250(2)	1-(2,6-Dimethylheptyl)-*p*-menthene-8(9)
12	278	$C_{20}H_{38}$	81(100), 95(91), 123(62), 278(21), 137(19)	1-[4(β,γ,γ-trimethyl-γ-valerolactonyl)]-*p*-menthene-8(9)
13	278	$C_{20}H_{38}$	81(100), 95(85), 68(76), 123(68), 278(26)	1-(2,7-Dimethyl-octyl)-*p*-menthene-8(9)
14	262	$C_{18}H_{30}O$	43(100), 84(80), 97(59), 125(42), 262(14)	Farnesyl acetone
15	256	$C_{16}H_{32}O_2$	73(100), 129(67), 213(42), 256(39), 227(19)	Ethyl-3,10-dimethyl-undecanoate
16	286	$C_{19}H_{26}O_2$	203(100), 218(19), 286(10), 243(5), 271(3)	Cannabidivarin
17	314	$C_{21}H_{30}O_2$	231(100), 314(25), 299(11), 271(10), 246(6)	$\Delta^{4(8)}$-*iso*-Tetrahydrocannabinol
18	328	$C_{21}H_{28}O_3$	246(100), 257(80), 300(64), 285(60), 328(36)	2-[5-(2-keto-*n*-pentyl)resorcinolyl]-*p*-mentha-1,8(9)-diene
19	314	$C_{21}H_{30}O_2$	231(100), 314(17), 299(6), 271(5), 246(3)	Cannabicyclol
20	312	$C_{17}H_{16}O_4$	149(100), 91(51), 206(29), 257(12), 312(6)	Benzyl butyl phthalate[a]
21	314	$C_{21}H_{30}O_2$	231(100), 314(8), 299(6) 271(2), 245(2)	Cannabichromene
22	282	$C_{19}H_{22}O_2$	267(100), 238(14), 282(11), 223(5), 209(3)	Cannabivarin
23	314	$C_{21}H_{30}O_2$	231(100), 246(12), 314(6), 299(3), 271(3)	Cannabidiol
24	314	$C_{21}H_{30}O_2$	299(100), 314(79), 231(66), 271(46), 258(22)	Δ^9-Tetrahydrocannabinol
25	310	$C_{21}H_{26}O_2$	295(100), 238(12), 310(10), 239(4), 251(4)	Cannabinol
26	380	$C_{27}H_{56}$	57(100), 71(88), 43(60), 351(5), 380(3)	Heptacosane
27	394	$C_{28}H_{58}$	57(100), 71(85), 43(55), 365(4), 394(3)	Octacosane
28	402	$C_{27}H_{46}O_2$	295(100), 402(87), 312(76) 231(48), 387(21)	1-[1-Methylcyclohex-3-enyl]-18-methyl-nonadeca-1,5,9-trien-4,5-diol
29	408	$C_{29}H_{60}$	57(100), 71(78), 85(64), 365(2), 408(2)	Nonacosane
30	408	$C_{29}H_{60}$	57(100), 295(19), 218(18), 393(17), 408(7)	9-Methyloctacosane
31	422	$C_{30}H_{62}$	57(100), 71(70), 85(62), 295(9), 422(1)	Triacontane
32	436	$C_{31}H_{64}$	57(100), 71(80), 85(63), 351(3), 436(2)	Hentriacontane
33	424	$C_{30}H_{48}O$	218(100), 203(63), 295(23), 424(15), 409(11)	6-Keto-Δ^{12}- or $\Delta^{13(18)}$-oleanene or -ursene
34	426	$C_{30}H_{50}O$	218(100), 203(54), 426(6), 295(5), 365(4)	α-Amyrin
35	466	$C_{32}H_{50}O_2$	218(100), 203(30), 466(17), 295(14), 390(9)	6-Hydroxy-23-aceto-Δ^{12}- or $\Delta^{13(18)}$-oleanene or -ursene
36	468	$C_{32}H_{52}O_2$	218(100), 203(43), 295(11), 468(8), 408(6)	α-, β-, or γ-Amyrin acetate
37	468	$C_{31}H_{48}O_3$	218(100), 203(35), 396(27), 295(20), 468(8)	6-Keto-10-nor-23-aceto-Δ^{12}- or $\Delta^{13(18)}$-oleanene or -ursene
38	426	$C_{30}H_{50}O$	408(100), 393(91), 302(69) 218(62), 426(18)	β- or γ-Amyrin

[a] Most likely a contaminant from a plastic container.

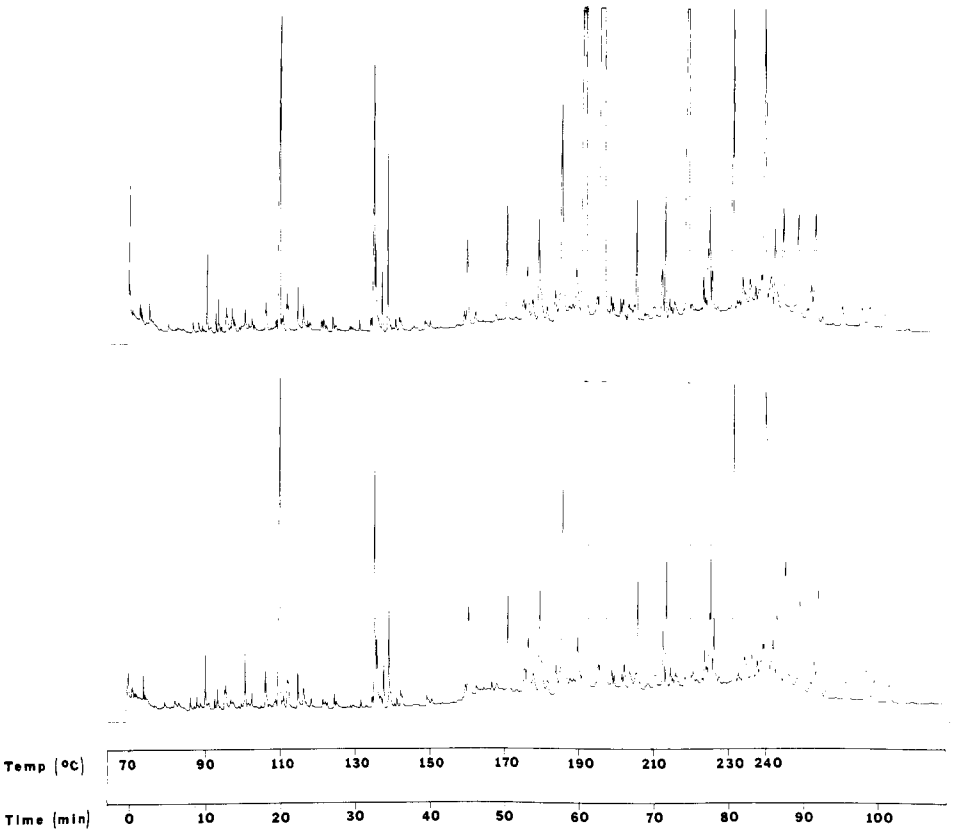

Figure 2. Chromatograms of two separately processed aliquots of identical material. Column: same as Figure 1

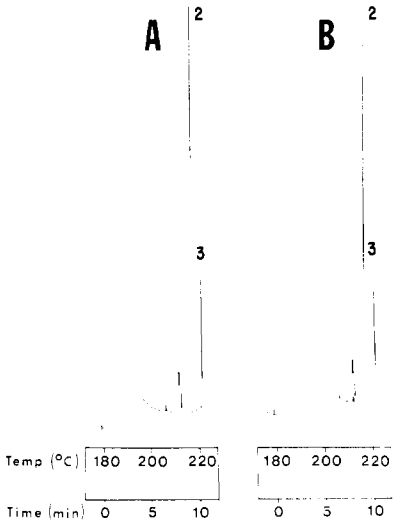

Figure 3. Chromatograms of identical injections of extracts of two different marijuana samples

(A) Colombian marijuana, (B) Mexican marijuana (different source than B and C of Figure 1); (1) Cannabidiol, (2) Δ^9-Tetrahydrocannabinol, (3) Cannabidiol. Column: same as Figure 1

(B) marijuana. It is obvious that one not knowing the origin of these samples could easily mistake them for being the same. On the other hand, there can be little doubt that different samples are dealt with when a more powerful fingerprinting method is used (Figure 4).

Many constituents of the recorded chromatographic profiles were identified through combined gas chromatography–mass spectrometry. Table I lists the obtained analytical data together with identified structures (see Figure 1 for peak numbers). The use of combined GC-MS is hardly necessary for a more rigorous fingerprinting. However, it is of wider interest to related research areas to establish which compounds may or may not be typical for various types of marijuana. Only cannabinoids can be considered truly "characteristic" constituents of the plant; yet, diagnostically important changes (as shown in this work) can be observed in lower terpenoids, alkanes, and triterpene regions of GLC profiles, even when such classes of compounds are commonly found in higher plants.

While only peaks 23, 24, and 25 were identified by comparing their respective spectra and retention times with those of authentic compounds and published spectra (*19, 20*), the identification of other cannabinoids through the similarity of their spectra and published chromatographic information (*21–24*), is considered straightforward. Likewise, peaks 1, 5, 20, 26, 27, 29, 31, and 32 provide easily recognizable mass-spectral features and their GC behavior correlates well with boiling points.

While it could be expected that 9-methyloctacosane should have shorter retention than the normal C_{29} alkane, the effect of branching position on melting point observed with C_{24} and C_{30} hydrocarbons (*25*) is consistent with the reversed order of elution observed in this work.

It should be explained that many structures, including some unusual compounds, contained in Table I were identified through the ab initio interpretation of their fragmentation patterns. Unavailability of both published spectra and authentic compounds has been the most serious problem. With only nominal-mass resolution available, these identifications must still be considered tentative. Unfortunately, the common identification methods (NMR- and/or IR-spectrometry) are presently compatible with neither the used concentration range, nor "on-line" investigation of capillary column effluents. Yet, we consider these tentative identifications important, since the classical approach of large-scale fractionation and positive spectral identifica-

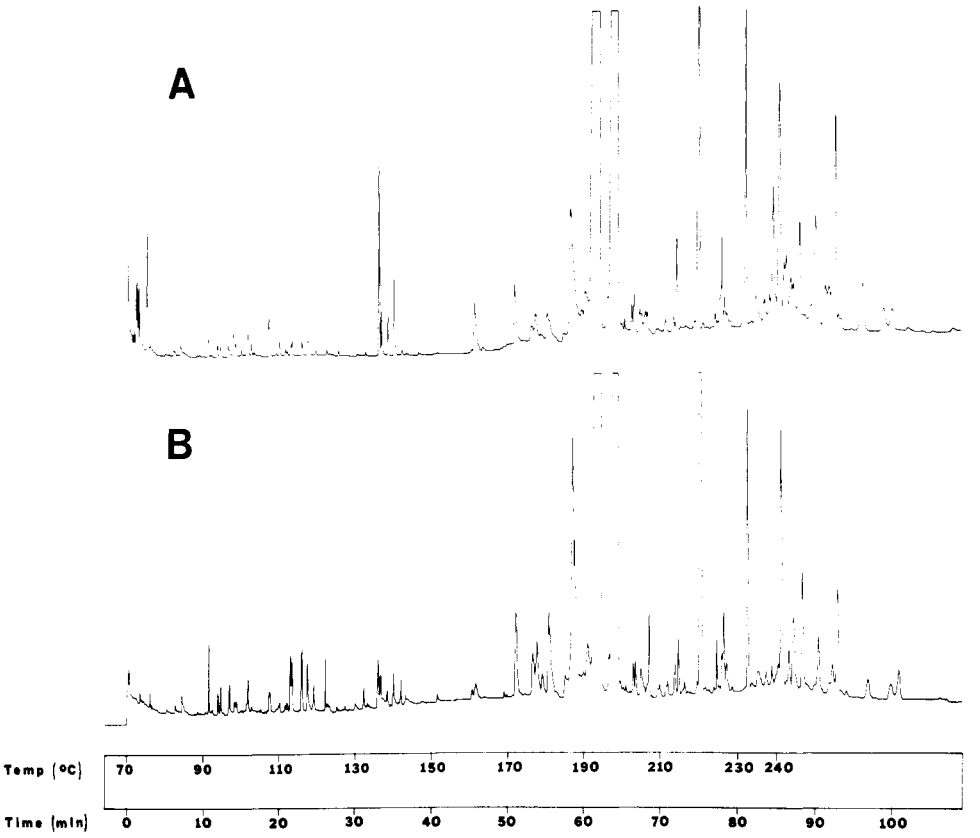

Figure 4. Capillary gas chromatograms of the cyclohexane fraction of the same samples as shown in Figure 2

(A) Colombian marijuana, (B) Mexican marijuana. Column: same as Figure 1

tion of isolated substances may present years of tedious research, if fruitful in some instances at all.

CONCLUSION

The fingerprinting method developed in this work has sufficient simplicity and reproducibility to be used for applications of forensic interest. Its specificity has shown a distinct advantage over the hitherto used methods. We suggest that the possibilities of correlation between chromatographic data and marijuana geographical origin be reconsidered in view of the presented results. Correlation studies may further be facilitated by use of a fully-automated gas-chromatographic system and advanced computational methods, similar to those being developed in our laboratory for biomedical profile comparisons (*26*).

Although the identification of profile constituents has not been of primary importance in this application, the value of combined capillary gas chromatography/mass spectrometry in marijuana-related research is clearly indicated.

ACKNOWLEDGMENT

Valuable discussions with Robert B. Forney of the Department of Toxicology, Indiana University Medical School, prior to initiation of this project, are greatly appreciated. We thank Michelle Franck for technical assistance and Paul Mahlberg of the Department of Plant Sciences, Indiana University, for his generous gift of Indiana-grown Mexican marijuana.

LITERATURE CITED

(1) A. B. Segelman, *J. Chromatogr.*, **82,** 151 (1973).
(2) T. W. M. Davis, C. G. Farmilo, and M. Osadchuk, *Anal. Chem.*, **35,** 751 (1963).
(3) A. Ohlsson, C. I. Abou-Chaar, S. Agurell, I. M. Nilsson, K. Olofsson, and F. Sandberg, *Bull. Narc.*, **23** (1), 29 (1971).
(4) A. N. Masoud and N. J. Doorenbos, *J. Pharm. Sci.*, **62,** 315 (1973).
(5) J. W. Fairbairn and J. A. Liebmann, *J. Pharm. Pharmacol.*, **26,** 413 (1974).
(6) C. W. Waller, *Pharmacol. Rev.*, **23,** 265 (1971).
(7) R. Phillips, R. Turk, J. Manno, N. Jain, and R. Forney, *J. Forensic Sci.*, **15,** 191 (1970).
(8) R. F. Turk, J. E. Manno, N. C. Jain, and R. B. Forney, *J. Pharm. Pharmacol.*, **23,** 190 (1971).
(9) C. E. Turner, K. W. Hadley, P. S. Fetterman, N. J. Doorenbos, M. W. Quimby, and C. Waller, *J. Pharm. Sci.*, **62,** 1601 (1973).
(10) C. E. Turner, K. W. Hadley, and P. S. Fetterman, *J. Pharm. Sci.*, **62,** 1739 (1973).
(11) L. Strömberg, *J. Chromatogr.*, **68,** 253 (1972).
(12) R. A. de Zeeuw, J. Wijsbeek, and T. M. Malingré, *J. Pharm. Pharmacol.*, **25,** 21 (1973).
(13) K. D. Bartle, M. L. Lee, and M. Novotny, *Int. J. Environ. Anal. Chem.*, **3,** 349 (1974).
(14) M. L. Lee, K. D. Bartle, and M. Novotny, *Anal. Chem.*, **47,** 540 (1975).
(15) M. Novotny and R. Farlow, *J. Chromatogr.*, **103,** 1 (1975).
(16) K. D. Bartle, L. Bergstedt, M. Novotny, and G. Widmark, *J. Chromatogr.*, **45,** 256 (1969).
(17) C. E. Turner and K. Hadley, *J. Pharm. Sci.*, **62,** 251 (1973).
(18) A. S. Curry, *J. Chromatogr. Sci.*, **12,** 529 (1974).
(19) H. Budzikiewicz, R. T. Alpin, D. A. Lightner, and C. Djerassi, *Tetrahedron*, **21,** 1881 (1965).
(20) U. Claussen, H.-W. Fehlhaber, and F. Korte, *Tetrahedrom*, **22,** 3535 (1966).
(21) R. A. de Zeeuw, J. Wijsbeek, D. D. Breimer, T. B. Vree, C. A. M. van Ginneken, and J. M. van Rossum, *Science (London)*, **125,** 778 (1972).
(22) T. B. Vree, D. D. Breimer, C. A. M. van Ginneken, and J. M. van Rossum, *J. Pharm. Pharmacol.*, **24,** 7 (1972).
(23) T. B. Vree, D. D. Breimer, C. A. M. van Ginneken, J. M. van Rossum, R. A. de Zeeuw, and A. H. Witte, *Clin. Chim. Acta*, **34,** 365 (1971).
(24) L. Strömberg, *J. Chromatogr.*, **63,** 39 (1971).
(25) Sir I. Heilbron, A. H. Cook, H. M. Bunbury, and D. H. Hey, "Dictionary of Organic Compounds", Oxford University Press, New York, 1965, pp 2970 (tetracosane), 3092 (triacontane), 233 (12-methyltricosane), 2263 (28-methyl-nonacosane), and 2337 (2-methyltricosane).
(26) M. L. McConnell and M. Novotny, *J. Chromatogr.*, **112,** 559 (1975).

RECEIVED for review July 17, 1975. Accepted October 9, 1975. This work was supported by Research Grant No. DA 00507-01 from the National Institute of Mental Health.