Mass Spectrometric Characterization of Cannabinoids in Raw *Cannabis sativa* L. Samples

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Qualitative characterization of *Cannabis sativa* L. (marihuana) is probably one of the most common tests done in forensic laboratories. The Duquenois-Levine color test (*1*) is very popular and effective in most cases. Since the test does not identify the presence of specific cannabinoids, an extraction/GC/MS analysis procedure is often used (*2–7*) in cases where specificity or quantitative information is needed. The latter method, nevertheless, is hindered by the laborious extraction process which also requires a large quantity of sample which is often not available.

This report describes a mass spectrometric method for the qualitative characterization of cannabinoids. The procedure requires only a few minutes of analysis time and a minute quantity (0.2 mg) of raw samples with minimum or no sample pretreatment. Yet, the method is so specific that no other test is needed for qualitative identification.

EXPERIMENTAL

A CEC (now owned by E. I. Du Pont, Wilmington, Del.) 21-104 mass spectrometer at Southern Illinois University was used for the preliminary study. Results reported here were obtained from an AEI (San Diego, Calif.) MS30 mass spectrometer at the University of Illinois at Chicago Circle. A typical experiment used 0.2 mg of pulverized sample which was introduced into the mass spectrometer via a direct-inlet-probe. The probe was heated solely by radiation and conduction from the source chamber which was maintained at 150 °C. The temperature of the probe was monitored. Spectra were obtained at various probe temperatures and ionization voltages.

Authentic Δ-1-tetrahydrocannabinol (Δ-1-THC), Δ-6-tetrahydrocannabinol (Δ-6-THC), cannabinol (CBN), and cannabidiol (CBD) were obtained from Applied Science Laboratories (State College, Pa.). Samples 1-L and 1-F are leaf and flower of a known *Cannabis sativa* L. plant. Sample 2-L is a "street" *Cannabis sativa* L. leaf of unknown source.

The Regression Subprogram in SPSS (*8*) was used for multiple regression analyses as described later.

RESULTS AND DISCUSSION

The combination of the thermal degradation/pyrolysis technique with mass spectrometry has recently been reviewed (*9, 10*). Two main differences exist between those methods and the method adopted here. First, heat was applied only to increase the vapor pressure of the compounds of interest; therefore, thermal degradation was kept at a minimum. Second, our electron impact source allowed us to observe the variations of fragmentation pattern at different energy levels. The previous methods thermally degrade the sample, and further fragmentations are minimized by use of a chemical ionization source (*9, 10*).

Two criteria are used to determine the presence of specific cannabinoids, and, therefore, to conclude the sample under examination is *Cannabis sativa* L.: (1) The parent peaks and major fragments of most known cannabinoids were sought in the spectrum. (2) Regression analyses were performed for peak height of samples on that of four controlled major cannabinoids. The latter analyses were done with data obtained at four different source energy levels to ensure the correlation is not accidental.

Table I. Qualitative Comparison on Major Mass Spectrometric Peaks of Controlled Cannabinoids and Raw *Cannabis sativa* L. Samples

	major m/e[a]	
sample	from literature	our results[b]
CBN	295, 310, 238, 223, 251, 231(11)	295, 310, 238
CBD	231, 246, 314, 299, 271, 258, 243(12)	231, 246, 314, 299, 258
Δ-1-THC	314, 299, 231, 271, 243, 258(13)	314, 299, 231, 295[c], 258, 193, 246, 310[c], 238[c]
Δ-6-THC	231, 314, 271, 258, 246, 193, 299, 243(12)	231, 314, 258, 271, 193, 246, 299, 243
1-L		314, 231, 299, 295, 271, 258, 243, 193, 246, 310, 238
1-F		314, 231, 299, 271, 295, 258, 243, 193, 246
2-L		231, 295, 314, 299, 271, 193, 246, 258, 243, 310, 238

[a] m/e peaks are listed in decreasing order of intensity. [b] Solid samples were introduced via a direct-inlet-probe. Spectra were obtained at 20 eV. [c] These peaks are believed to be fragments of CBN which derived from Δ-1-THC through thermal conversion (see Figure 1).

Table I summarizes the characteristic mass number (m/e) for each of the four authentic cannabinoids and three *Cannabis sativa* L. samples. Despite the difference in the sample introduction techniques, our results on pure cannabinoids are comparable to those reported previously (*11–13*).

Observations of characteristic cannabinoid peaks, as shown in Table I, generally provide sufficient information in concluding the sample under investigation is *Cannabis sativa* L. A definite quantitative conclusion is based on the correlation between the sample spectrum and that of controlled cannabinoids. The mass spectrum of a compound is significantly changed as the source ionization energy varies (*17–19*). The characteristic ionization-potential vs. peak-height plot of a *Cannabis sativa* L. sample reflects the characteristic combination of cannabinoids in that sample. However, these peak intensities should correlate well with those of cannabinoids under corresponding conditions. This correlation is tested as described below.

The mass spectrum produced by a *Cannabis sativa* L. sample is the sum of its individual components. The characteristic contribution of each component is revealed by the

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Table II. Mass Spectrometric Data of Four Controlled Cannabinoids and Three Raw *Cannabis sativa* L. Samples[a]

		m/e										
sample	E[b]	314	310	299	295	271	258	246	243	238	231	193
CBN	20	0.0	20.0	0.0	100.0	0.0	0.0	0.0	0.0	10.5	0.0	0.0
	18	0.0	19.8	0.0	100.0	0.0	0.0	0.0	0.0	15.7	0.0	0.0
	16	0.0	14.4	0.0	100.0	0.0	0.0	0.0	0.0	13.6	0.0	0.0
	14	0.0	14.8	0.0	100.0	0.0	0.0	0.0	0.0	13.4	0.0	0.0
CBD	20	14.1	0.0	5.5	0.0	0.0	4.6	32.2	0.0	0.0	100.0	14.4
	18	12.2	0.0	2.9	0.0	0.0	1.4	29.5	0.0	0.0	100.0	10.4
	16	9.9	0.0	3.6	0.0	0.0	3.0	29.3	0.0	0.0	100.0	11.6
	14	10.6	0.0	1.7	0.0	0.0	2.3	27.7	0.0	0.0	100.0	9.1
Δ-1-THC	20	100.0	7.3[c]	92.1	30.2[c]	49.9	26.8	8.7	28.1	3.9[c]	70.9	13.9
	18	100.0	5.1	95.2	21.9	48.4	26.1	7.4	31.3	2.8	74.7	11.0
	16	100.0	4.8	92.2	17.8	50.0	27.3	9.2	28.2	4.5	76.3	11.5
	14	100.0	6.3	87.6	23.0	48.1	29.4	8.6	27.9	3.6	74.8	14.9
Δ-6-THC	20	52.9	0.0	9.0	0.0	33.8	35.8	17.1	4.3	0.0	100.0	29.4
	18	70.9	0.0	9.7	0.0	38.6	38.6	19.0	4.7	0.0	100.0	30.7
	16	72.1	0.0	11.0	0.0	36.7	35.0	20.0	4.6	0.0	100.0	30.6
	14	69.4	0.0	10.1	0.0	36.9	35.5	21.3	5.2	0.0	100.0	29.5
1-L	20	100.0	11.4	68.7	52.4	43.0	25.4	15.6	23.5	7.8	97.7	19.9
	18	100.0	13.8	71.4	54.6	43.9	24.0	15.3	23.5	9.7	100.0	14.3
	16	65.4	11.3	41.9	44.2	30.0	15.2	21.2	16.6	6.0	100.0	17.3
	14	61.0	10.4	36.2	37.7	25.8	16.4	24.5	16.7	5.0	100.0	17.0
1-F	20	100.0	0.0	68.7	25.3	40.4	24.7	14.6	21.7	0.0	90.9	21.7
	18	100.0	0.0	68.1	28.4	39.7	23.4	16.3	23.4	0.0	92.2	27.7
	16	77.1	7.5	50.4	25.4	25.8	15.4	20.8	16.3	7.9	100.0	25.8
	14	56.0	4.3	36.1	25.5	20.4	12.5	22.1	10.3	8.2	100.0	21.2
2-L	20	40.9	9.8	33.0	41.2	25.3	15.3	22.4	14.0	7.7	100.0	23.5
	18	35.4	8.1	25.5	37.8	18.0	11.1	25.8	14.4	9.0	100.0	23.4
	16	25.5	6.8	18.0	30.8	14.6	9.2	24.0	7.1	6.4	100.0	20.0
	14	20.4	7.2	13.2	29.1	14.8	9.5	21.7	7.8	7.4	100.0	16.1

[a] Peak intensities in the body of the table were normalized to 100. [b] Source ionization energy. [c] These peaks were originated from CBN which was derived from Δ-1-THC through thermal conversion as shown in Figure 1. The exclusion of these peaks for regression analysis caused an expected increase in the regression coefficient of the CBN term.

Table III. Multiple Regression of Y on X at Various Source Ionization Energy

		Δ-1-THC		CBD		CBN		Δ-6-THC				
sample	E[a]	coef[b]	S.E.[c]	coef	S.E	coef	S.E	coef	S.E.	R^2[d]	S.E.	ctn[e]
1-L	20	0.78	0.051	0.17	0.092	0.31	0.051	0.28	0.11	0.99	4.4	−1.7
	18	0.76	0.041	0.20	0.062	0.41	0.042	0.26	0.070	0.99	3.6	−1.7
	16	0.43	0.041	0.46	0.059	0.36	0.038	0.20	0.068	0.99	3.3	0.33
	14	0.39	0.038	0.53	0.054	0.27	0.034	0.17	0.064	0.99	2.9	1.7
1-F	20	0.79	0.058	0.11	0.11	0.049	0.58	0.30	0.12	0.99	5.1	−4.1
	18	0.70	0.075	0.088	0.11	0.14	0.077	0.36	0.13	0.98	6.6	−2.1
	16	0.50	0.078	0.40	0.11	0.16	0.074	0.22	0.13	0.97	6.4	0.87
	14	0.38	0.052	0.59	0.074	0.16	0.047	0.13	0.088	0.99	4.0	0.76
2-L	20	0.26	0.048	0.61	0.087	0.30	0.048	0.16	0.099	0.98	4.2	3.1
	18	0.18	0.044	0.73	0.067	0.28	0.045	0.079	0.076	0.99	3.9	5.0
	16	0.12	0.039	0.79	0.056	0.26	0.037	0.086	0.066	0.99	3.2	2.7
	14	0.082	0.053	0.82	0.075	0.24	0.047	0.065	0.089	0.99	4.1	3.4

[a] Source ionization energy. [b] Regression coefficient. [c] Standard error. [d] Coefficient of determination. [e] Constant term of the regression equation.

spectrum obtained from each pure standard. At one particular ionization energy, the relationship between the peak height of the raw *Cannabis sativa* L., and that of pure cannabinoids is formulated as follows:

$$\begin{aligned} Y_1 &= X_{11}r_1C_1 + X_{12}r_2C_2 + \ldots + X_{1m}r_mC_m \\ Y_2 &= X_{21}r_1C_1 + X_{22}r_2C_2 + \ldots + X_{2m}r_mC_m \\ &\vdots \\ Y_n &= X_{n1}r_1C_1 + X_{n2}r_2C_2 + \ldots + X_{nm}r_mC_m \end{aligned} \quad (1)$$

where X_{nm} is the relative intensity of the peak, named as n, of compound m, C_m is the concentration of compound m in the raw sample under examination, r_m is the relative sensitivity factor of compound m, and Y_n is the observed intensity of the peak, named as n, in the raw *Cannabis sativa* L. sample.

The sensitivity factors and the concentration terms of the above equations are combined, and these equations and those formulated at other source energy levels are expressed as:

$$[Y_j = \sum_{i=1}^{m} X_{ji}B_i \quad (j = 1, 2, \ldots n)]_E \quad (2)$$

where $B_i = r_iC_i$, and E is the source ionization energy.

The conventional multiple regression method was chosen (*20*) for the treatment of mass spectrometric data listed in Table II. Regression information listed in Table III indicates that, in all cases, more than 97% of the data obtained from raw *Cannabis sativa* L. can be correlated to that of controlled cannabinoids. This gives a quantitative indication that the mass spectrometric data of samples 1-L, 1-F, and 2-L listed in Table II are contributed by cannabinoids. It is, therefore,

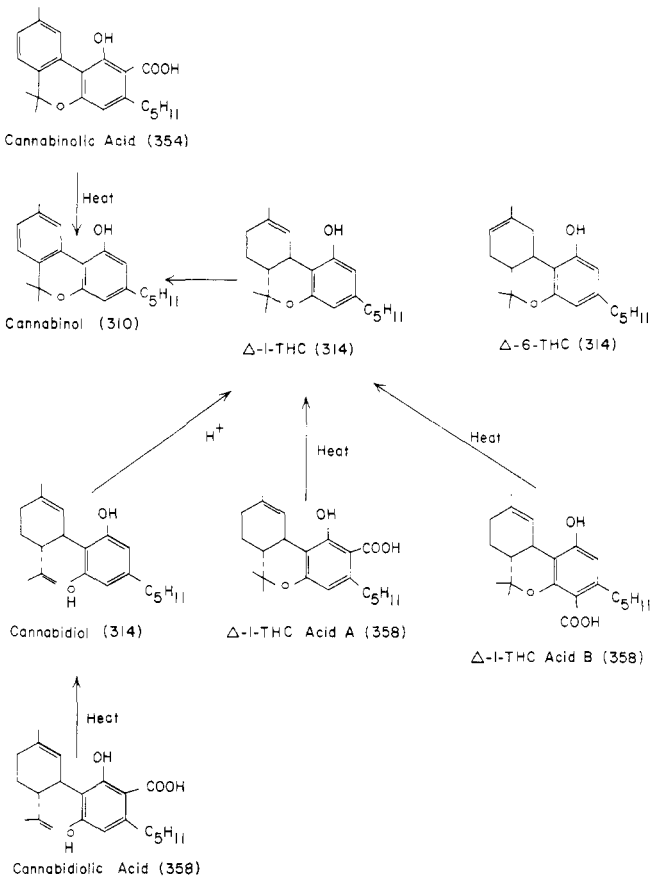

Figure 1. Chemical structure, molecular weight, and possible interconversions of major cannabinoids (*14–16*)

Table IV. Multiple Regression of Modified *Canabis sativa* L. Mass Spectra

		regression coefficients					
sample	E^a	Δ-THC	CBD	CBN	Δ-6-THC	R^{2b}	ctn^c
1-L	20	0.27	0.86	0.26	−0.51	0.30	28
	18	0.40	0.94	0.25	−0.66	0.39	30
	16	0.23	0.99	0.23	−0.43	0.63	20
	14	0.18	1.0	0.15	−0.44	0.68	21
1-F	20	0.22	0.88	-	−0.58	0.21	30
	18	0.28	0.94	-	−0.68	0.29	33
	16	0.24	1.1	-	−0.60	0.54	26
	14	0.16	1.1	0.043	−0.49	0.72	21
2-L	20	0.077	0.85	0.28	−0.12	0.79	14
	18	0.066	0.96	0.23	−0.21	0.89	15
	16	0.046	0.97	0.21	−0.13	0.93	9.5
	14	0.28	0.95	0.21	−0.092	0.95	8.5

[a] Source ionization energy. [b] Coefficient of determination. [c] Constant term of the regression equation.

concluded that the samples under examination are *Cannabis sativa* L. Variations of regression coefficients at different source voltages are contributed by the following factors: (1) The relative sensitivity factors (*r*'s in Equation 1) may have varied; (2) changes of *C* terms (Equation 1) as a result of fluctuations in the vaporization of cannabinoids during the period spectra of various source conditions were obtained; and (3) experimental error and/or deviation from the model chosen for regression (*20*). Further study on the change of relative sensitivity factors at different source conditions is in progress.

Spectra obtained from mixture other than cannabinoids may not contain all of those mass units listed in Table II, or at least with different relative intensities. Correlation of these spectra with controlled cannabinoid data should result in significant deviation of constant term from zero, low value of coefficient of determination, or even negative correlation coefficient for some terms. To test this hypothesis, the relative intensities of mass units 314 and 310 of all three samples (Table II) were exchanged and the same regression analyses were performed. Regression results tabulated in Table IV indicated that good correlation cannot be achieved even with this relatively minor manipulation.

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Degassed-Solvent Reservoir for High Performance Liquid Chromatography

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Degrassing is one of the effective preliminary treatments of mobile phases required for successful experiments of high performance liquid chromatography (HPLC). Solvent degassing is effective in preventing bubbles from forming in a detector. Especially in cases where dissolved gases are reactive to mobile or stationary substances, or to some particular

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