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TECHNICAL NOTE

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Possible Characterization of Samples of *Cannabis sativa* L. by Their Carbon Isotopic Distributions

To achieve the ultimate goal in "individualizing" drug samples, qualitative and quantitative composition determinations are commonly used [*1–5*]. Based on quantitative analysis of major cannabinoids, pharmaceutical scientists [*2,3*] have concluded that *Cannabis sativa* L. can be chemically categorized into drug and fiber types. These investigators [*2,3*] further established that the phenotype of a plant is determined by the genetic origin of the seed, and the location of cultivation is irrelevant. On the other hand, according to geochemists, environmental factors seem to control isotope distribution in plants of the same species. Although the category of a plant is controlled by the adopted carbon fixation pathway [*6–8*], the carbon isotope ratio $^{13}C/^{12}C$ within a category reflects the environmental conditions such as humidity, temperature, photoperiod, and isotope composition of ambient carbon dioxide [*7–14*] in which the plant has grown. It is therefore interesting to analyze the isotopic distribution in *Cannabis sativa* L. of different origins grown under various conditions for possible characterization of seed origin and location of cultivation.

Experimental Procedure

The basic analytical procedure was as follows. Twenty milligrams of sample was pulverized and mixed with 100 mg of cupric oxide. The mixture was then combusted inside a quartz tube that composed part of a vacuum line. The combustion was carried up to 900 ± 5 °C and maintained at this temperature for 10 min. Platinum gauze was used to assist the completion of combustion [*15*]. The gas generated was recycled through the quartz tube with a Toepler pump during the entire combustion process. At the end, the carbon dioxide generated was trapped with liquid nitrogen after passing through an acetone/dry ice trap. The carbon dioxide collected was analyzed for its 45 and 44 peaks with a Nuclide 6-60-RMS (double inlet-double collector) isotope ratio mass spectrometer. Data obtained were corrected for instrumental errors (mixing and tailing) and ^{17}O contents [*16*]. Carbon dioxide generated from Grenville calcite was used as the working standard; results are reported in relation to PDB standard in parts per mil ($\delta\ ^{13}C$):

$$\delta\ ^{13}C = \left| \frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{standard}} - 1 \right| \times 1000$$

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Samples ME(3)-MI-M-L (A) and RU(2)-MI-M-L (B) in Table 1 are leaves of male *Cannabis sativa* L. plants grown in Mississippi, with seeds originating from Mexico (third generation) and Russia (second generation), respectively. The seed origins of samples NO-OA-NO-L (C), NO-NA-M-L (D), and NO-NA-M-F (E) were not certain. Sample C is a leaf sample collected in Oak Park, Ill., and D and E are leaf and flower samples, respectively, of a plant grown indoors in Naperville, Ill. Sample NO-NA-F-L (F) is a leaf sample of a female plant grown under conditions identical to D and E, and presumably with seed of the same origin.

Results and Discussion

Results from Samples D and E indicated that the carbon isotope distributions in flower and leaf are different, as predicted. This difference is due to the variation of chemical compositions in these parts of the plants. It has been shown [*17*] that the $^{13}C/^{12}C$ ratio does vary in different chemical fractions.

Ambient temperature does not correlate with the results obtained. To the first approximation the ^{13}C content in the ambient air seems to control the isotopic composition of the plant. The ^{13}C content in the samples analyzed decreased in the order A, B (Mississippi), C (Oak Park), D, and F (indoor). This order coincides with the order of the ^{13}C content in the ambient air under which these plants were grown. Since a heater that used natural gas containing less-than-usual amounts of ^{13}C was used in the greenhouse, the carbon dioxide inside the greenhouse would be the lightest. The reabsorption of carbon dioxide derived from plants confined in the greenhouse might have led to further depletion in ^{13}C content [*18*]. Oak Park is located in the metropolitan Chicago area. It seems reasonable to assume that the carbon dioxide in Oak Park is lighter than that in Mississippi [*19*]. Results obtained from Samples A and B indicate the possibility of ^{13}C content variation in plants of different origins. However, a definite conclusion can be drawn only with additional investigations. Whether the difference in Samples D and F is due to the sex difference is not certain as the origin of the seeds is not well established.

While results indicated variations of $^{13}C/^{12}C$ ratios in *Cannabis sativa* L. samples, these variations are within a rather narrow range. A complete individualization of *Cannabis sativa* L. samples based on this technique alone is unrealistic. However, the addition of

TABLE 1—*Ratio of* $^{13}C/^{12}C$ *in samples of* Cannabis sativa *L.*

Sample	Sample Code[a]	Run	$\delta^{13}C$	Average
A	ME(3)-MI-M-L	1	−28.11	−27.99
		2	−27.82	
		3	−28.05	
B	RU(2)-MI-M-L	1	−28.85	−28.80
		2	−28.76	
		3	−28.79	
C	NO-OA-NO-L	1	−29.85	−30.25
		2	−30.64	
D	NO-NA-M-L	1	−31.64	−31.72
		2	−31.79	
E	NO-NA-M-F	1	−34.15	−34.13
		2	−34.26	
		3	−33.97	
F	NO-NA-F-L	1	−32.82	−32.80
		2	−32.79	
		3	−32.78	

[a] Samples are described in the text.

this approach to the much-used qualitative and quantitative analyses would increase the chance of success in sample differentiations.

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