Mississippi-Grown *Cannabis sativa* L.: Preliminary Observation on Chemical Definition of Phenotype and Variations in Tetrahydrocannabinol Content *versus* Age, Sex, and Plant Part

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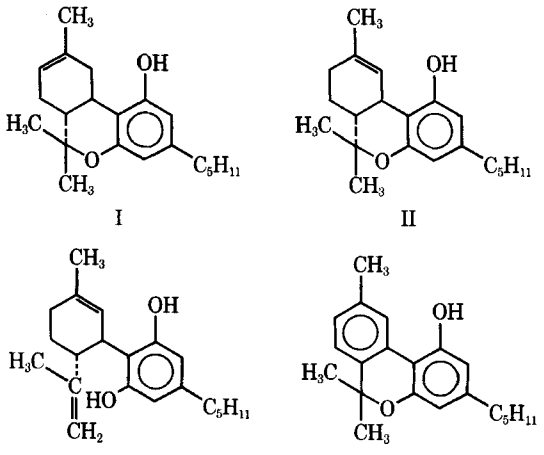
Abstract □ Nine strains of *Cannabis sativa* L. (marijuana) were grown for research by the University of Mississippi. The seeds for these strains were obtained from Iowa, Minnesota, Mexico, Turkey, Italy, France, and Sweden. The cannabinoid content was determined using GLC, and the material was divided into two chemical phenotypes according to cannabinoid content. These phenotype categories are used to differentiate between drug-type and fiber-type *Cannabis sativa*. In addition, the (−)-Δ⁹-*trans*-tetrahydrocannabinol content was determined for both male and female plants, various plant parts, and a Turkish variety during various stages in its growth.

Keyphrases □ *Cannabis sativa*, nine strains—phenotype definition, tetrahydrocannabinol content *versus* age, sex, plant part □ Tetrahydrocannabinol, *Cannabis sativa*—content compared by age, sex, plant part □ Phenotypes, chemical—drug and fiber *Cannabis sativa* □ GLC—analysis, tetrahydrocannabinol

It is generally known that two types of marijuana exist. One type is used principally for drug purposes, while the other type is used mainly for its fiber. So far, there are no botanical methods by which the two types of plant material may be distinguished. The pharmacological effects usually associated with marijuana are produced by (−)-Δ⁸-*trans*-tetrahydrocannabinol[1] (I) and (−)-Δ⁹-*trans*-tetrahydrocannabinol (II) (1, 2). The other main cannabinoids present are cannabidiol (III) and cannabinol (IV). Cannabidiol is generally considered to be the precursor in the metabolic pathway to (−)-Δ⁹-*trans*-tetrahydrocannabinol (3, 4). It has not been found to have any psychotomimetic activity. According to Levine (5), cannabinol is the degradation product of (−)-Δ⁹-*trans*-tetrahydrocannabinol. Grlić (3)[2] differentiated samples of *Cannabis sativa* on the basis of the cannabinoids by means of combined spectrophotometric methods. He classified his samples into various "ripening" stages. Test animals may be used to approximate the tetrahydrocannabinol content of marijuana (6, 7).



GLC was used for the quantitation of the samples reported in this paper. Studies and analyses of *Cannabis sativa*, as reported here, indicated that marijuana may be classified into two chemical phenotypes according to cannabinoid content.

METHODS

Samples used were either grown in Mississippi or received from the National Institute of Mental Health (NIMH) or various individual sources. The seeds for the plants grown in Mississippi in 1968 and 1969 were obtained from Mexico, Turkey, Italy, France, Sweden, and wild stands in Iowa and Minnesota. This material was harvested and manicured[3] so that analyses could be made according to the sex of the plant and the various plant parts as well as the source of the seeds. The seeds[4], roots, various sizes of stems, leaves, bracts, and male flowers were separated and analyzed for cannabinoid content. To eliminate cannabinoids from outside sources, the seeds were washed in chloroform prior to extraction. Then they were crushed and extracted according to the procedure described here. The Turkish variety was collected after sexual differentiation at intervals during the growing season so the presence of cannabinoids could be traced through the growth of the plant.

The extraction was basically that used by Lerner (8) as modified by those working with analysis of tetrahydrocannabinol, both natural and synthetic. An analysis consisted of 1 g. of material in 40 ml. of chloroform. This solution was kept in the refrigerator and shaken at 10-min. intervals for 1 hr. The plant material was then removed by filtration, and the chloroform was removed *in vacuo* at 40°. The remaining residue was dissolved and filtered in 25 ml. ethanol (5 × 5-ml. aliquots). After evaporation under reduced pressure, the residue was dissolved in 1 ml. of ethanol containing a known concentration of 4-androstene-3,17-dione as the internal standard. One microliter of this solution was injected into the chromatograph.

Analyses were performed using Beckman GC-5 and GC-45 gas chromatographs equipped with flame-ionization detectors and operated isothermally at 210° with an inlet temperature of 230°. The columns were 0.41-cm., 3.04-m. (0.125-in., 10-ft.) stainless steel packed with 2% OV-17 (phenyl methyl silicone) on 100/120 mesh Gas Chrom Q[5]. Nitrogen was used as the carrier gas at a flow of 30 ml./min.[6]. Peak area measurements were made using the method of

[1] Naturally occurring (−)-Δ⁸-*trans*-tetrahydrocannabinol was first isolated by R. L. Hively, W. A. Mosher, and F. W. Hoffman, *J. Amer. Chem. Soc.*, **88**, 1832(1966). For a summary of the comparison of natural and synthetic materials, see R. Mechoulam, *Science*, **168**, 1159 (1970).

[2] Grlić proposed a coding system containing 32 coded numbers, each corresponding to one of the parameters he used. Each sample has at least six "coded characteristics," which are necessary to classify marijuana into one of his "ripening" stages: "unripe, intermediate, ripe, overripe, and altered."

[3] Manicured material is devoid of seeds and large stems. This was accomplished by passing the material through a 10-mesh sieve.

[4] The seeds of marijuana are really fruits which serve as seeds.

[5] Obtained from Applied Science Laboratories, State College, Pa.

[6] Under the conditions of analysis, all cannabinoid acids would be converted to their respective phenols.

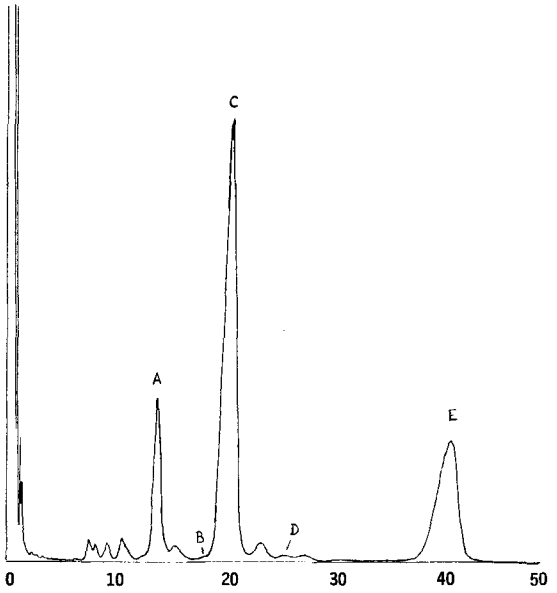


Figure 1—*Chromatogram of 1969 male Mexican marijuana grown in Mississippi. Phenotype I. Peaks: A = cannabidiol, B = (−)-Δ⁸-trans-tetrahydrocannabinol, C = (−)-Δ⁹-trans-tetrahydrocannabinol, D = cannabinol, and E = internal standard.*

peak height times width at half-height. The peak area of each cannabinoid was compared with the peak area of the internal standard; using the appropriate correction factors, the cannabinoid concentration then was determined. Through NIMH, synthetic (−)-Δ⁸-*trans*-tetrahydrocannabinol[7] and (−)-Δ⁹-*trans*-tetrahydrocannabinol[7] and cannabidiol[8] and cannabinol[8] were obtained.

RESULTS AND DISCUSSION

Phenotype—Recently, Grlić classified *Cannabis sativa* into five "ripening" groups (*Footnote 2*) (3). Also an attempt was made to classify the plant into fiber and drug types on the basis of the cannabinoid acids as well as the phenols[9]. The following ratio is proposed as a means of classifying marijuana into chemical phenotypes:

$$\text{phenotype ratio} = \frac{\%\,(-)\text{-}\Delta^9\text{-}trans\text{-tetrahydrocannabinol} + \%\,\text{cannabinol}}{\%\,\text{cannabidiol}} \quad \text{(Eq. 1)}$$

Grlić proposed a similar ratio: (tetrahydrocannabinol + cannabinol)/(cannabidiol acid + cannabidiol), using UV and IR spectral data, along with other ratios with which to classify marijuana into "ripening" groups. This ratio does not take into account the acids of cannabinol and tetrahydrocannabinol. The phenotype ratio makes use of data obtained from a method in which the cannabinoid acids were converted into their corresponding cannabinoid phenols, similar to the results of storage or smoking.

Analytical work indicates that some plant materials have a very high cannabidiol content while others have a high (−)-Δ⁹-*trans*-tetrahydrocannabinol content. Limited experience in handling the individual cannabinoids shows (−)-Δ⁹-*trans*-tetrahydrocannabinol to be the least stable. According to Levine (5), loss of potency of marijuana is accompanied by conversion of (−)-Δ⁹-*trans*-tetrahydrocannabinol to cannabinol. This leads to the suggestion that (−)-Δ⁹-*trans*-tetrahydrocannabinol + cannabinol would approximate the (−)-Δ⁹-*trans*-tetrahydrocannabinol content, irrespective of degradative changes.

[7] From Arthur D. Little, Inc., Cambridge, Mass.

[8] From Research Triangle Institute, Research Triangle Park, N. C.

[9] Toffoli *et al.* (9) attempted, by a complicated alkaline extraction and subsequent GLC analysis, to classify *Cannabis sativa* into fiber and biologically active types. They concluded that in the fiber type the cannabidiol was present largely as the acid. From the acid analyses in this study, it was concluded that the acids themselves are not indicative of the type of plant material but rather of the age after harvesting, the drying conditions, or the storage of the plant material. The fiber and drug types can be ascertained by a simple analysis and comparison of the ratio of the cannabinoids, without regard to their acids as such.

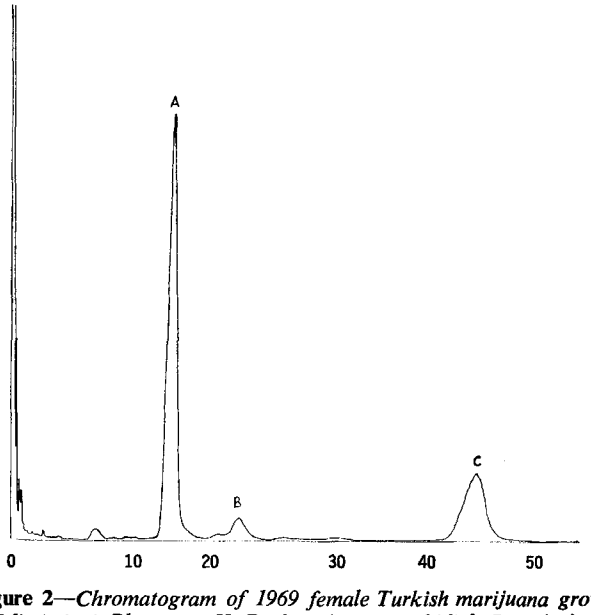


Figure 2—*Chromatogram of 1969 female Turkish marijuana grown in Mississippi. Phenotype II. Peaks: A = cannabidiol, B = (−)-Δ⁹-trans-tetrahydrocannabinol, and C = internal standard.*

Marijuana samples with phenotype ratios greater than 1.0 are classified into Phenotype I, which represents the biologically active type of marijuana. Samples having ratios less than 1.0 are classified into Phenotype II or the fiber-type marijuana. Figure 1 is a chromatogram produced by an extract of Phenotype I marijuana; Fig. 2 was produced by an extract of Phenotype II marijuana.

The data in Table I are the results of various studies of marijuana. The table shows the extensiveness with which the phenotype system applies to the marijuana plant. For instance, this system classifies all the various plant parts, including those found underground, of each plant or variety into the same phenotype. All parts of the Mexican plant are Phenotype I material, while all the Turkish plant parts are in Phenotype II. This also is the case for the male and female plants of each species. A survey of the Turkish plant showed that the plant, when analyzed during various stages of its growth, was always Phenotype II. The phenotype of one variety remains the same each year. For example, samples of Mexican marijuana of 1968 and 1969 were analyzed and both were found to be Phenotype I. The place of growth of the plant material makes no difference in the phenotype classification. The Minnesota and Iowa samples grown both at their origin and in Mississippi are Phenotype II. From the data, therefore, the phenotype of one variety of marijuana remains the same regardless of the plant part, sex, age, year, or place of growth of the sample analyzed.

The only exceptions to this classification system were samples having unknown histories. These unknown samples were either confiscated or supplied without protocol, and all could have contained mixtures of various types of marijuana. All samples of known origin and history fit into the phenotype classification system. Therefore, as a preliminary observation, it seems that the chemical phenotype classification proposed here is a relatively simple and useful means of distinguishing between the drug and fiber types of marijuana.

Male *versus* Female—It was believed previously that the male plant contained little or no resin and, by definition, marijuana consisted of the female parts of *Cannabis sativa* L., the male plants being grown mainly for fiber (10). When marijuana was last classified as an official drug in 1936, it was defined as "the dried flowering tops of the pistillate plants" (11). Recently, Valle *et al.* (6) showed, through pharmacological studies, that male marijuana is as potent as the female. However, there have been no published data as to the actual (−)-Δ⁹-*trans*-tetrahydrocannabinol content of male and female marijuana[10]. Data from this study (Table I) show that the male

[10] Private communication with Dr. Stig Agurell, Department of Pharmacognosy, Faculty of Pharmacy, Uppsala University, Stockholm, Sweden, confirms the data that male and female marijuana contains roughly similar amounts of cannabinoids.

Table I—GLC Analysis and Phenotype Classification of Various Samples of Marijuana[a]

Sample	From	Percent Cannabidiol	Percent (−)-Δ^9-*trans*-Tetrahydrocannabinol	Percent Cannabinol	Phenotype Ratio	Phenotype
Mexican, 1968:						
Female	UM	0.075	1.0	0.54	21	I
Male	UM	0.32	1.2	0.59	5.6	I
Mexican, 1969:						
Female	UM	0.12	1.4	0.073	12	I
Male	UM	0.40	1.5	0.070	3.9	I
Immature	UM	0.063	0.60	0.002	9.5	I
Mexican female plant parts:						
Bracts	UM	0.15	3.7	0.18	26	I
Small leaves	UM	0.085	1.4	0.051	17	I
Seeds	UM	t	0.01	0.01	$\gg$1.0	I
Mexican male plant parts:						
Flowers	UM	0.88	1.6	0.078	1.9	I
Leaves	UM	0.079	1.0	0.047	13	I
Stems	UM	0.055	0.89	0.076	18	I
Turkish, 1968:						
Female†	UM	$\sim$6 $\times$ Δ^9-THC	0.059	0.023	N.Q.	II
Male	UM	0.24	0.0070	t	0.029	II
Turkish, 1969:						
Female	UM	1.7	0.18	0.062	0.14	II
Turkish female plant parts:						
Bracts†	UM	$\sim$15 $\times$ Δ^9-THC	0.37	0.038	N.Q.	II
Leaves†	UM	$\sim$5 $\times$ Δ^9-THC	0.32	0.088	N.Q.	II
Stems:						
1-mm. diameter	UM	0.19	0.02	t	0.11	II
2–4 mm.	UM	0.03	0.007	t	0.23	II
10–15 mm.	UM	0.003	t	t	$<$1.0	II
Roots	UM	0.015	0.0020	0.00074	0.18	II
Seeds	UM	0.0087	0.00057	t	0.066	II
Turkish male at various weeks after planting:						
8 weeks	UM	0.11	0.02	0.02	0.36	II
11 weeks	UM	0.21	0.03	0.04	0.33	II
15 weeks	UM	0.28	0.02	0.02	0.14	II
18 weeks	UM	0.53	0.04	0.01	0.094	II
Turkish female at various weeks after planting:						
8 weeks	UM	0.15	0.02	0.02	0.27	II
11 weeks	UM	0.21	0.03	0.04	0.33	II
15 weeks	UM	0.28	0.02	0.03	0.18	II
18 weeks	UM	0.87	0.04	0.04	0.092	II
19 weeks	UM	1.00	0.05	0.02	0.07	II
Minnesota, 1968:						
1	Minn.	0.77	0.073	0.028	0.13	II
2	Minn.	1.2	0.074	0.016	0.075	II
Minnesota, 1969	UM	0.71	0.054	0.0095	0.089	II
Minnesota female plant parts:						
Bracts	UM	1.3	0.054	0.0033	0.044	II
Leaves	UM	1.0	0.043	t	0.043	II
Iowa, 1968	Iowa	0.95	0.061	0.026	0.092	II
Des Moines, 1968	Des Moines, Iowa	1.2	0.071	0.010	0.068	II
Illinois male, 1968*	Dr. Susiana and Dr. Dunbar, Samford Univ.	0.26	1.1	0.085	4.6	I
Seized marijuana*	Dr. L. Way, Univ. of California	0.88	0.084	t	0.095	II
Carmagnola, 1968	UM	1.2	0.32	0.085	0.34	II
Fibranova, 1969	UM	1.55	0.11	0.040	0.097	II
Unknown*	Dr. A. Yuwiler, Vet. Hosp., Los Angeles, Calif.	0.71	0.077	t	0.11	II
Confiscated cigarette*:						
a	Dr. H. Isbel,	1.08	0.15	0.049	0.18	II
b	Univ. of Kentucky	0.48	0.51	0.10	1.3	I
Monophyllous bracts	UM	6.1	0.5	t	0.082	II
Charas tincture*	Dr. C. C. Pfeiffer, Princeton	3.8	1.4	4.0	1.4	I
Unknown—laboratory grown*	UM, grown by Dr. Walter	2.1	0.19	t	0.091	II
Hashish cake and powder*	Athens, Greece, Dr. M. Fink, New York Univ.	9.8	2.1	3.5	0.57	II
Red oil or marijuana extract distillate	Research Triangle Institute	0.88	10	3.5	15	I
NIMH 1 (confiscated)*	NIMH	0.095	0.58	0.37	10	I

Table I—(*Continued*)

Sample	From	Percent Cannabidiol	Percent (−)-Δ^9-*trans*-Tetrahydrocannabinol	Percent Cannabinol	Phenotype Ratio	Phenotype
NIMH 12*	NIMH	1.0	0.052	0.020	0.072	II
USP fluid extract* of *Cannabis sativa*, manufactured by H. K. Mulford Co., 40 years old	Dr. K. Redman, South Dakota State Univ.	2.7	0.43	5.2	2.1	I
Thailand QCD-65472, 1969	Dr. R. Forney, School of Medicine	0.16	2.2	t	14	I
Thailand QCD-65169, 1969	Indianapolis, Ind.	0.11	1.3	t	12	I
Carmagnola (Italy), 1969	UM	1.4	0.37	0.077	0.32	II
Fibranova (Italy), 1969	UM	1.6	0.11	0.04	0.094	II
Unknown*		0.19	0.025	0.018	0.23	II
Turkish extract, 1969	UM	28	1.4	t	0.050	II

[a] UM = University of Mississippi. † = cannabidiol peak offscale, roughly estimated. * History unknown. t = trace. ~ = approximately. N.Q. = not quantitative.

and female of the same variety contain similar amounts of (−)-Δ^9-*trans*-tetrahydrocannabinol.

Various Plant Parts—A GLC survey of the various plant parts showed that the parts decrease in (−)-Δ^9-*trans*-tetrahydrocannabinol content in the following order: bracts, flowers, leaves, smaller stems, larger stems, roots, and seed. All plant parts contain cannabinoids. The data from this study are reported in Table I.

Plant Growth—From the preliminary study of the growth of the Turkish plant, it was found that young plants contain cannabinoids. No other conclusions were made. Some of the data from this study appear in Table I.

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