CONSTITUENTS OF *CANNABIS SATIVA* L., XIV: INTRINSIC PROBLEMS IN CLASSIFYING CANNABIS BASED ON A SINGLE CANNABINOID ANALYSIS

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It is generally accepted that *Cannabis* consists of only one species, *Cannabis sativa* L., but because of a long history of cultivation in a wide range of diverse geographical locations, *Cannabis* has evolved into many different variants with pronounced morphological and chemical differences. Because of these differences, several classification procedures have been developed.

Grlić (1) first classified samples of *Cannabis* into various "ripening types" according to predominant cannabinoids: "unripe," predominantly cannabidiolic acid (CBDA); "intermediate," cannabidiol (CBD); "ripe," (−)-*trans*-Δ^9-tetrahydrocannabinol Δ^9-THC); and "over ripened," cannabinol (CBN). Waller (2, 3), using quantitative chemical data, defined *Cannabis* as fiber or drug type. In the drug type Δ^9-THC+CBN/CBD is greater than 1.0 and in the fiber type less than 1.0. The ratio of cannabinoids is characteristic of the genetic strain of *Cannabis*, but is dependent on the stage of growth, sex of plant, part of plant analyzed, and to some extent on the conditions of cultivation. Small and Beckstead (4) subdivided *Cannabis* into phenotype I (>0.3% Δ^9-THC; <0.5% CBD), phenotype II (>0.3% Δ^9-THC; >0.5% CBD), and phenotype III (<0.3% Δ^9-THC). A fourth phenotype was represented by the plants from northeastern Asia which consistently showed trace amounts (about 0.05%) of cannabigerol monomethylether (CBGM).

Phillips *et al.* (5) indicated a cyclic peaking of cannabidiol throughout the growing season of a variant growing wild in Indiana. A cyclic pattern of cannabinoids (CBD, Δ^9-THC, and CBN) was observed by Turner *et al.* (6) in studies of a Mexican variant grown in Mississippi. They reported that the content of cannabinoids varied in a rhythmic fluctuation and was a function of time of day and age of plant parts at sampling.

In the past decade, 228 strains of seeds from different geographical locations originating from 61 countries have been grown in Mississippi. Among these, 85 variants and some of their descendants were analyzed weekly in order to investigate the growth profile of three major naturally occurring cannabinoids: cannabichromene (CBC), CBD, and Δ^9-THC. Leaves were randomly collected from six different plants of the same variant at the same time and day of each week. The age of the plants were recorded from dates of planting. Samples from young, male and female plants were collected. Samples taken from each plant were pooled, air dried, and manicured to remove stems by passing the dry leaves through a 14-mesh sieve (6). The resulting marihuana samples were quantitatively analyzed for ten cannabinoids using the procedure described by Turner *et al.* (7); namely Δ^9-THC, CBC, CBD, CBN, CBGM, (−)-*trans*-Δ^8-tetrahydrocannabinol (Δ^8-THC), (−)-*trans*-Δ^9-tetrahydrocannabivarin (Δ^9-THCV), cannabidivarin (CBDV), cannabigerol (CBG), and cannabicyclol (CBL).

TABLE 1. *Growth profile of cannabinoid content of* Cannabis, *Indian variant: Cannabinoids expressed as percent by dry weight.*[a]

Age[b]	Δ^9-THC	Δ^8-THC	Δ^9-THCV	CBL	CBN	CBC	CBD	CBDV	CBG	CBGM	Waller	Small
Male:											Type	Phenotype
19	1.13	0.09	0.20	0.06	0.02	0.31	1.16	0.22	0.13	0.00	fiber	II
20	0.80	0.07	0.21	0.01	t[c]	0.18	0.69	0.32	0.09	0.01	drug	II, IV
21	1.91	0.01	0.67	0.02	0.01	0.32	0.01	0.07	0.11	t	drug	I
22	0.24	0.25	0.03	t	0.07	0.09	0.81	0.34	0.03	0.01	fiber	III, IV
23	0.66	0.02	0.13	t	0.09	0.17	1.45	0.20	0.07	t	fiber	II
24	2.05	0.02	0.43	0.01	t	0.31	0.23	0.04	0.09	0.00	drug	I
25	0.44	0.03	0.10	t	t	0.22	0.98	0.31	0.08	0.00	fiber	II
Female:												
19	1.61	0.05	0.20	t	0.02	0.28	1.11	0.07	0.02	0.00	drug	II
20	1.24	0.06	0.40	0.02	t	0.17	0.56	0.24	0.09	0.02	drug	II, IV
21	1.06	0.03	0.23	0.01	0.10	0.21	1.10	0.30	0.03	0.00	drug	II
22	1.54	0.34	0.32	0.01	0.03	0.04	0.37	0.09	0.09	t	drug	I
23	1.99	0.03	0.74	0.03	0.05	0.20	0.02	0.04	0.12	0.02	drug	I, IV
24	1.05	0.02	0.24	0.02	t	0.26	0.55	0.09	0.02	0.00	drug	II
25	0.75	0.04	0.41	t	t	0.11	0.68	0.28	0.11	0.00	drug	II

[a]Seed code IN-K received from Dr. C. K. Atal, 1-27-1975.
[b]Age in weeks after planting on 4-14-1976.
[c]t=less than 0.009%.

Using the analytical data, we found that a single *Cannabis* variant, depending on the age and sex of the plants, could be classified as either drug or fiber type following Waller's chemical classification (2, 3). In addition, a single variant could fall in any of the four phenotypes used by Small and Beckstead (4) depending on its stage of growth.

To illustrate this point we have used an Indian variant normally thought to be a drug type and have confined our data to reflect plants that can be sexually differentiated (see table 1); however, cannabinoid ratios in young plants also vary. From the data in table 1 it is clear that a single analysis using Δ^9-THC, CBD, and CBN is at best a variant indicator. For example, at week 23 the Indian male plants are fiber type according to Waller's method and phenotype II according to Small's method, whereas, the female plants are drug type and phenotype I and IV, respectively.

Cannabinoids, of which more than 60 are known (8), fluctuate in a cyclic pattern; *Cannabis* plant material is thereby placed in different chemical classifications according to age, sex, and plant part. It is therefore impossible to use a single analysis or even two analyses to fully classify a *Cannabis* variant. However, analysis of a single sample will provide useful data on the crude drug. It is therefore concluded from our studies of large populations of different *Cannabis* variants grown in Mississippi, USA, since 1968 that, to date, all classification systems proposed for *Cannabis* are only valid for the particular sample analyzed and not for the variant. Moreover, all previous chemical classification systems based on CBD are of limited value since CBC has been erroneously identified as CBD (9). This was the case in the analytical procedures reported in references 2–4.

Currently we are investigating the possibility of using a formula as shown below to classify different variants of *Cannabis*.

$$\text{Phenotype} = \frac{\Delta^9\text{-THC}+\Delta^9\text{-THCV}+\text{CBN}+\Delta^8\text{-THC}+\text{CBL}}{\text{CBC}+\text{CBD}+\text{CBDV}+\text{CBG}+\text{CBGM}}$$

This formula takes into account quantifiable homologs and separates the cannabinoids according to their ring structure or system. The result of this investigation will be the subject of a forthcoming communication.

ACKNOWLEDGMENTS

This work was supported by the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, and the National Institute on Drug Abuse, Contract #HSM-42-70-109. We wish to thank Dr. C. K. Atal, Regional Research Laboratory, Jammu, Kashmir, India, for his assistance.

Received 11 December 1978.

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