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Cannabis. XII.[1] Variations of Cannabinoid Contents in Several Strains of *Cannabis sativa* L. with Leaf-age, Season and Sex

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A quantitative analysis procedure for tetrahydrocannabinolic acid, cannabichromenic acid, cannabidiolic acid and cannabigerolic acid monomethyl ether was established by gas chromatography and by a combined gas chromatography–preparative thin-layer chromatography method using cholestane as an internal standard.

The variations of cannabinoid contents with leaf-age, season and sex were investigated in three kinds of "physiological varieties" of *Cannabis sativa* L., the Mixican, the Minamioshihara No. 1 and the CBDA strain.

Keywords——Moraceae; Cannabis; Cannabinoid; quantitative analysis; tetrahydrocannabinolic acid; cannabidiolic acid; cannabichromenic acid; cannabigerolic acid monomethyl ether

Introduction

The cannabinoids in *Cannabis sativa* L. have been investigated both quantitatively and qualitatively in detail.[3–13] However, the cannabinoid profile from the seedling to reproductive stages of distinct varieties of *Cannabis sativa* L. has not yet been determined.

This paper describes the variations of the cannabinoid contents with leaf-age, season and sex in three kinds of "physiological varieties" of *Cannabis sativa* L.: the Mexican, the Minamioshihara No. 1 and the CBDA strains.

Results and Discussion

Assay System

All cannabinoids exist as the corresponding acids such as cannabinoid acid.[14] The cannabinoid acids can be completely decarboxylated to produce neutral cannabinoids and analyzed by gas–liquid chromatography (GLC).[11] For quantitative analysis, calibration plots were prepared using an internal standard. Cholestane was used as the internal standard in the analysis of the following compounds: cannabidiolic acid (CBDA), cannabichromenic acid

1) Part XI: Y. Shoyama, H. Hirano, and I. Nishioka, *J. Labelled Compd. Radiopharm.*, **14**, 835 (1978). This work was presented at the 94th Annual Meeting of the Pharmaceutical Society of Japan, 1974.
2) Location: *3-1-1 Maidashi, Higashiku, Fukuoka, Japan.*
3) T.W. Davis and C.G. Farmilo, *Anal. Chem.*, **35**, 751 (1963).
4) U. Claussen, W. Borger, and F. Korte, *Ann. Chem.*, **693**, 158 (1966).
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(CBCA), tetrahydrocannabinolic acid (THCA), and cannabigerolic acid monomethyl ether (CBGAM). The calibration plots were linear, thus establishing quantitative recovery (Fig. 1).

The dried Cannabis leaves were extracted with benzene, and the resulting extract was dried, then dissolved in acetone. THCA, CBCA, and CBGAM were analyzed by GLC.

As reported previously,[4] it is impossible to separate cannabidiol (CBD) and cannabichromene (CBC) by GLC without derivatization, so the trimethylsilyl ether derivatives were subjected to GLC analysis. Unfortunately, this method failed to give quantitative results so a combination of GLC and preparative thin-layer chromatography (PLC) had to be employed for the analysis of CBDA and CBCA. Crude benzene extracts were completely decarboxylated by heating at 150° for 10 min[15] and then analyzed by GLC and PLC. The recovery rates of CBC and CBD in PLC were 84.5% and 83.4%, respectively.

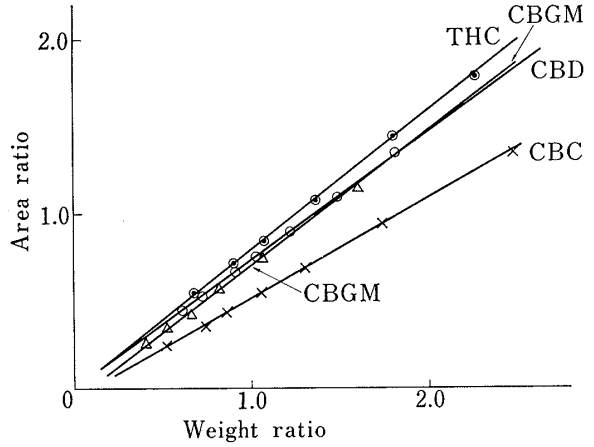

Fig. 1. Calibration Plot for Tetrahydrocannabinol, Cannabidiol, Cannabichromene and Cannabigerol Monomethyl Ether

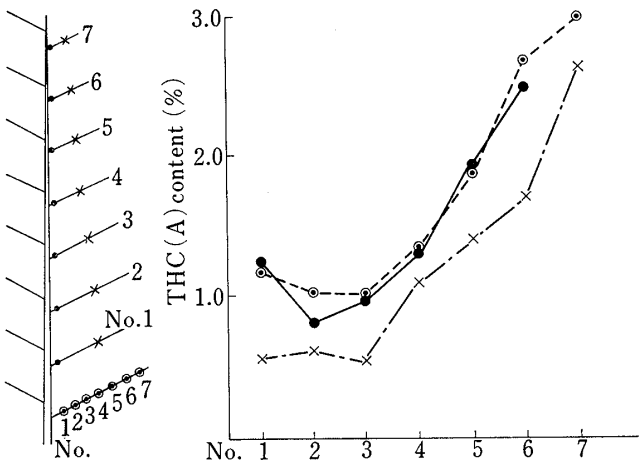

Fig. 2. Variation of Tetrahydrocannabinolic Acid Content with Leaf-age

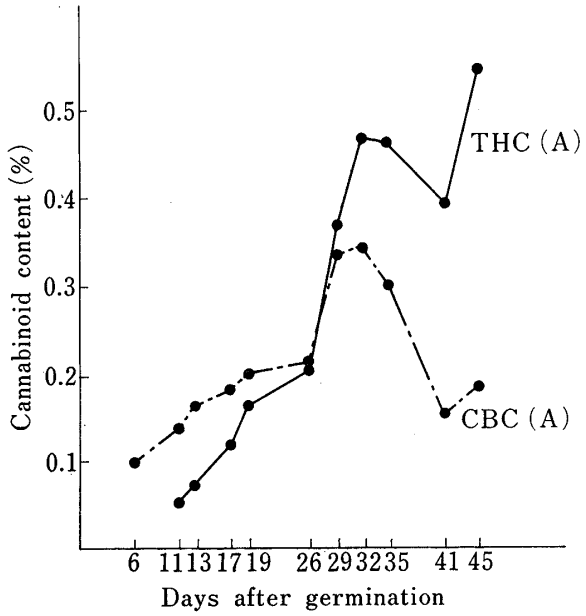

Fig. 3. Seasonal Variation of Cannabinoid Contents in the Mexican Strain

——— THC(A), —·—·— CBC(A).

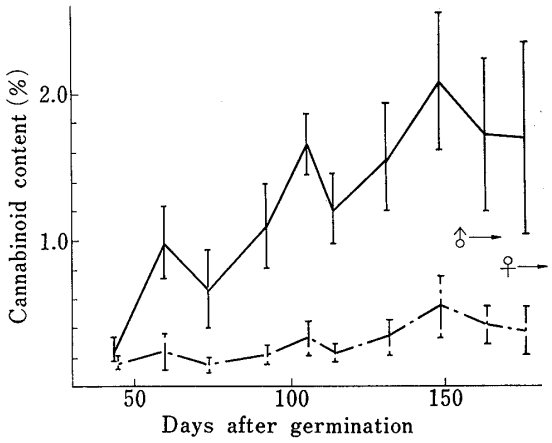

Fig. 4. Seasonal Variation of Cannabinoid Contents in the Mexican Strain

——— THC(A), —·—·— CBC(A).
Flowering: ♂ ♀.

15) Y. Shoyama, R. Oku, T. Yamauchi, and I. Nishioka, *Chem. Pharm. Bull.*, **20**, 1927 (1972).

For the Mexican and Minamioshihara No. 1 strains containing CBCA, THCA and CBGAM, the GLC method was used; for the CBDA strain containing CBDA and CBCA, a GLC–PLC combination method was used.

Variation of Cannabinoid Contents with Leaf-age

Figure 2 shows the differences in THCA content in the Mexican strain with respect to different sites on the leaf at the flowering stage. It shows clearly that the upper portion of the leaf contains a larger amount of THCA than the lower portion. In addition, from a comparison between sites No. 1 and No. 7 it is evident that younger leaves contain a larger amount of THCA than older leaves.

Since it is evident that there are significant variations in the amount of THCA, depending on the age of the leaves, an intermediate site (No. 4) was used for the investigation of seasonal and sexual variations of cannabinoid contents.

Variation of Cannabinoid Contents with Season

1) Figures 3 and 4 show the seasonal changes of cannabinoid contents in the Mexican strain. In the 6-day-old seedling, CBCA was the only cannabinoid present. THCA appeared at 11 days and rapidly increased in amount to become the predominant cannabinoid at 29 days. CBCA increased for 32 days after germination and then decreased during the next 10 days. The overall cannabinoid content increased steadily as the plant aged.

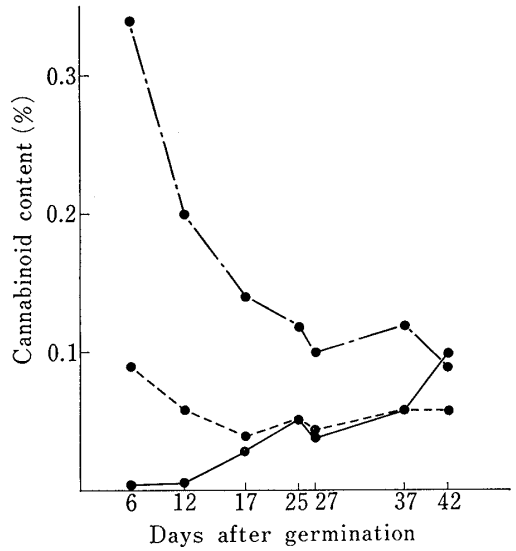

Fig. 5. Seasonal Variation of Cannabinoid Contents in the Minamioshihara No. 1 Strain

——— THC(A), —·—·— CBC(A), ------- CBG(A)M.

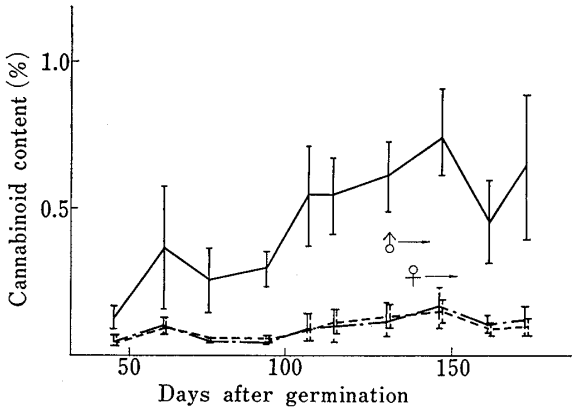

Fig. 6. Seasonal Variation of Cannabinoid Contents in the Minamioshihara No. 1 Strain

——— THC(A), —·—·— CBC(A), ------- CBG(A)M. Flowering: ♂ ♀.

2) The effect of season on the cannabinoid contents in the Minamioshihara No. 1 strain is shown in Figs. 5 and 6. The content of CBCA in 6-day-old seedlings of the Minamioshihara No. 1 strain was approximately 3 times greater than that of the Mexican strain, but then the CBCA content decreased rapidly for 21 days. The peak of THCA content occurred later in the Minamioshihara No. 1 strain than in the Mexican strain. Like the Mexican strain, the overall level of CBCA, THCA and CBGAM in Minamioshihara No. 1 increased steadily as the age of the plant increased.

3) Figs. 7 and 8 show the seasonal variation in the CBDA strain. Although the CBCA content decreased over the first two months, it remained relatively constant thereafter. On the other hand, the CBDA content increased gradually throughout plant growth. The

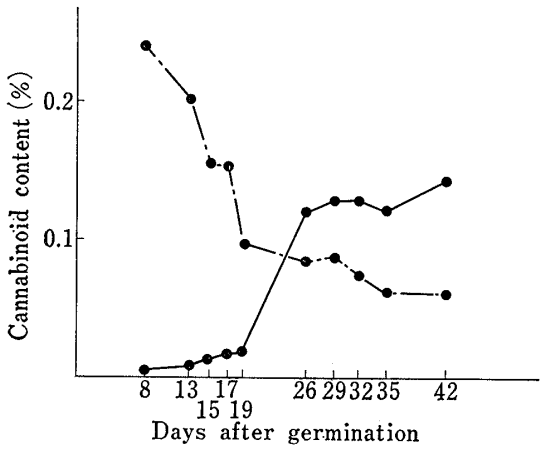

Fig. 7. Seasonal Variation of Cannabinoid Contents in the CBDA Strain
——— CBD(A), —·—·— CBC(A).

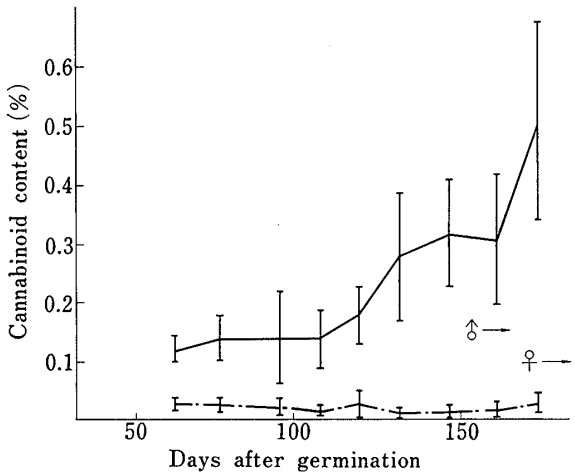

Fig. 8. Seasonal Variation of Cannabinoid Contents in the CBDA Strain
——— CBD(A), —·—·— CBC(A).
Flowering: ♂ ♀.

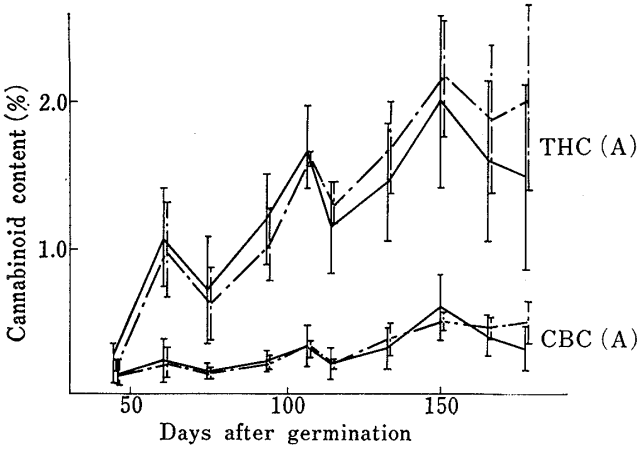

Fig. 9. Variation of Cannabinoid Contents in the Mexican Strain with Sex
———♀, —·—·—♂.

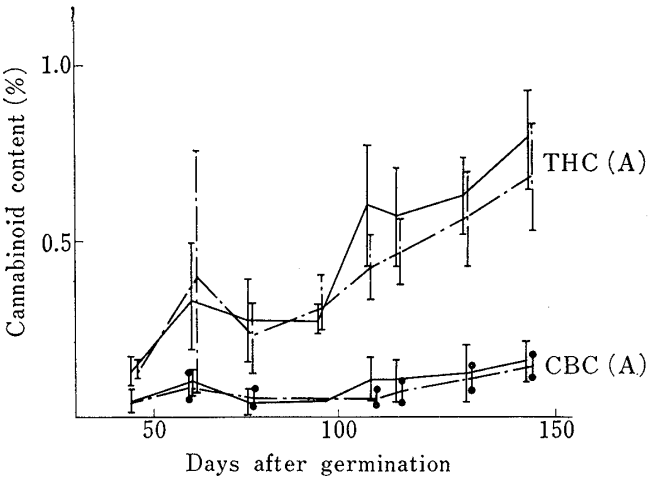

Fig. 10. Variation of Cannabinoid Contents in the Minamioshihara No. 1 Strain with Sex
———♀, —·—·—♂.

total cannabinoid content of the CBDA strain was about one-quarter of that of the Mexican strain.

Variation of Cannabinoid Contents with Sex

The effect of sex on cannabinoid contents was examined in the Mexican (Fig. 9) and the Minamioshihara No. 1 (Fig. 10) strains. These results confirm the previous data, showing no significant differences in cannabinoid contents[9] between the male and female plants.

Experimental

Plant Material——The Mexican strain: This is a typical THCA strain and contains THCA as a major cannabinoid, with CBCA as a minor cannabinoid. The Minamioshihara No. 1 strain: This is a typical domestic strain in Japan and contains THCA together with CBGAM[16] as major cannabinoids. The CBDA strain: This strain was obtained by selection from a Japanese domestic strain between 1967 and 1972.

16) Y. Shoyama, T. Yamauchi, and I. Nishioka, *Chem. Pharm. Bull.*, **18**, 1327 (1970).

This strain contains CBDA as a major cannabinoid and CBCA as a minor cannabinoid: THCA is not present. The details of selection of this strain will be published elsewhere.

Seeds of all strains were sown on 22nd March 1972 at this University and individual specimens were collected periodically between March and October, 1972. Young plants were obtained from seedlings in the greenhouse of this University in 1972.

Gas Chromatography——Apparatus: Shimadzu 5A gas chromatograph. Conditions: Column 1.5% SE-52, glass column (2.25 m×4 mm); column temp., 240°; detector temp., 250°; carrier, N_2 (25 ml/min), H_2 (50 ml/min), air (500 ml/min). Retention times (min): CBD(A) and CBC(A), 3.50; CBG(A)M, 4.00; THC(A), 4.50; cholestane, 11.50.

Preparation of Calibration Plots——Cholestane (64.4 mg) was dissolved in a small amount of hexane and diluted to 50 ml with acetone (1.292 mg/ml). A neutral cannabinoid of known concentration was dissolved in hexane and diluted with acetone to give a standard solution. Cholestane solution (0.1 ml) and neutral cannabinoid solution (0.1 ml) were combined and 2 μl of the mixture was injected into the gas chromatograph. The ratios of peak areas (cholestane: neutral cannabinoid) were determined from taking the peak half-widths. Calibration plots (Fig. 1) were obtained for separate cannabinoid preparations at the following concentrations. THC (mg/ml): 0.875, 1.167, 1.401, 1.751, 2.334, 2.918, 3.502. CBD (mg/ml): 0.790, 0.948, 1.185, 1.361, 1.579, 1.895, 2.236. CBC (mg/ml): 0.607, 0.958, 1.117, 1.341, 1.676, 2.235, 3.352. CBGM (mg/ml): 0.518, 0.671, 0.829, 1.036, 1.382, 2.073.

Determination of the Standard Deviation——The standard deviation (σ) of THC analysis in the Mexican strain was determined from the following data (10 runs): THC (%): 1.95, 1.94, 1.94, 1.91, 1.96, 1.95, 1.90, 1.96, 1.95, 1.96 (average; 1.94). CBD (%): 0.54, 0.52, 0.54, 0.56, 0.54, 0.55, 0.54, 0.56, 0.54, 0.53 (average; 0.54). THC (3.88 mg) $\sigma=0.04$ mg ($\sigma/m\times100=1.0\%$), CBC (1.8 mg) $\sigma=0.02$ mg ($\sigma/m\times100=1.8\%$). This method proved to be useful and reliable for the quantitative analysis of cannabinoids.

Determination of Recoveries of CBC and CBD on PLC——Kieselgel G (Merck, 20×20 cm) PLC plates were developed with benzene. CBD (102 μg) was recovered by scraping off the appropriate band, extracting the powder with MeOH (1.5 ml) and evaporating the solution to dryness *in vacuo*. The CBD obtained was redissolved in acetone (known volume) and analyzed (5 runs) by GLC. The recoveries were as follows for CBD (μg): 86.2, 88.4, 87.5, 81.9, 85.2. In the case of CBC, 117.1 μg was used and the recoveries were determined as described for CBD. The amounts (μg) of CBC recovered were 90.0, 88.7, 91.5, 90.3 and 92.2.

Extraction and Analysis——GLC Method: The dried powder (200 mg) of leaves was extracted with benzene (20 ml) at 60° for 3 hr. The benzene was evaporated off *in vacuo* and the residue was redissolved in acetone (0.5—2.0 ml). An aliquot (2 μl) was injected into the GLC apparatus and the content was calculated from the calibration plots in conjunction with the following equation:

$$\text{content } (\%) = \frac{1.292\times W\times V}{M}\times 100$$

W: weight ratio for cholestane
V: volume of acetone (ml)
M: weight of dried leaves (mg)

GLC-PLC Method: The leaves, in the form of dried powder (400 mg), were extracted with benzene (40 ml) at 60° for 3 hr and the benzene extract was heated at 160° for 10 min. The decarboxylated product was analyzed by PLC using benzene as a solvent. The appropriate band was scraped off then extracted with MeOH (15 ml). The extract was redissolved in acetone (0.5—2.0 ml) and analyzed as described previously.