optimum to lower pH values and this will result in increased overlap of the theoretical and experimental optimum ranges.

DISCUSSION

Many authors have recommended (3) a pH range of 7–7.5 for the extraction of amine–IX complexes with chloroform or other organic solvents. The experimental results of these studies (Table I) indicate that the best pH range for these extractions is from 5.2 to 6.4 for 1:1 complexes (I, II, IV, and VI) and from 3.0 to 5.8 for 1:2 (amine–dye) complexes (III, V, VII, and VIII). The theoretical results (Table IV) indicate a best pH range of 5.6–6.8 for 1:1 complexes and of 4.2–6.4 for 1:2 complexes. The best pH range was determined from the maximum absorbance value ±5% for the experimental data and from the maximum theoretical extraction ±2% for the computer data. Since experimental data are not as precise as theoretical data, a wider range was allowed on experimental values. Both the experimental and the theoretical data (Tables I and IV) show that the arbitrarily reported pH range of 7–7.5 (3) is not the optimum for a maximum sensitivity in the assay technique. Nevertheless, sometimes it may be preferable to sacrifice the sensitivity in favor of eliminating the interferences from other ingredients such as in the biological systems. At too low pH values (about 3–4.6), the chances of forming hard-to-break emulsions are greater than at higher pH values. The blank absorbance value against pure chloroform is zero above a pH value of 6.4. At lower pH values, the blank increases with a decrease in the pH, becoming significantly high (0.04–0.05) below pH 5.

It appears that a pH range of about 5.2–6.4 is the best for the extraction of amine–IX complexes. For 1:2 (amine–dye) complexes, the lower value (5.2) should be preferred.

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Mississippi-Grown *Cannabis sativa* L. III: Cannabinoid and Cannabinoid Acid Content

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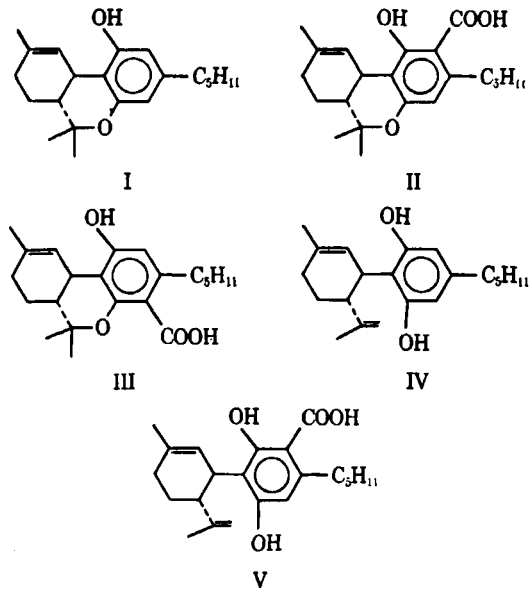
Abstract □ A procedure for the assay of acidic and nonacidic cannabinoids qualitatively and quantitatively by chemical fractionation prior to TLC and GLC analyses is reported. Various samples of foreign and domestic, wild and cultivated marijuana were analyzed. It was demonstrated that cannabinoids occur largely as acids, that these acids undergo decarboxylation upon storage, and that plants vary significantly in their cannabinoid composition due to heredity.

Keyphrases □ *Cannabis sativa* L.—analysis, acidic and nonacidic cannabinoids by chemical fractionation prior to GLC and TLC, hereditary plant composition and effect of storage on cannabinoid content □ Cannabinoids, acidic, nonacidic—analysis, chemical fractionation prior to GLC and TLC, hereditary plant composition, decarboxylation during storage □ Marijuana—analysis, acidic and nonacidic cannabinoids by chemical fractionation prior to GLC and TLC, hereditary plant composition, and effect of storage on cannabinoid content

The major cannabinoids and cannabinoid acids that occur naturally in the various strains of *Cannabis sativa* L. (marijuana) plants included in this investigation are Δ^9-tetrahydrocannabinol (I), its two corresponding acids Δ^9-tetrahydrocannabinoic acid A (II) and Δ^9-tetrahydrocannabinoic acid B (III), cannabidiol (IV), and its corresponding acid cannabidiolic acid (V) (1–3). It has been established that I is the principal psychotomimetic compound in marijuana (4). When marijuana is smoked, II and III undergo decarboxylation and are inhaled as I (5, 6). This is one reason why marijuana exerts higher activity when smoked than when eaten.

GLC is being used for the quantitative analysis of marijuana (7–9), but these procedures do not distinguish between I and its respective acids. The acids decarboxylate rapidly to I in the injection port. Thus, the data determined by GLC analysis represent I plus its two respective acids.

The Department of Pharmacognosy at the University of Mississippi is growing *C. sativa* L. in order to supply analyzed marijuana and marijuana extracts to the National Institute of Mental Health (NIMH) for



distribution to researchers who, in turn, will study the effects of these materials on animals and humans. It is essential that analytical methods distinguish between cannabinoids and cannabinoid acids in these research materials. This report includes the description of the first such procedures developed and the results of a comparative study of marijuana produced from several strains of *C. sativa* L.

EXPERIMENTAL

Materials—Mexican, Turkish, Swedish, Italian (Carmagnola, Fibranova), and French (Fibriman) *C. sativa* L. seeds were obtained in the spring of 1968 through NIMH. Marijuana produced from the plants grown from these seeds in the drug plant garden of the University of Mississippi in 1968 and 1969 was utilized in this study, along with samples of marijuana collected in September 1968 from wild plants growing in Iowa and Minnesota.

Extraction and Fractionation—One gram of plant material, finely manicured by passing through a 1-mm. sieve to remove seeds and stems and to reduce leaves, flowers, and bracts to small particles, was extracted by shaking overnight with 10 ml. of 95% ethanol. The ethanol was removed *in vacuo* at room temperature. The residue was dissolved in 20 ml. of a 1:1 mixture of petroleum ether and an aqueous solution of 2% sodium hydroxide and 2% sodium bisulfite. Cannabinoid acids dissolve in sodium hydroxide but not in sodium bicarbonate or sodium carbonate solutions. The sodium hydroxide solution does not extract the nonacid cannabinoids under these conditions, and they remain in the petroleum ether. Other workers (2, 10) reported similar results and attributed these observations to steric hinderance or to chelation between the carboxyl and neighboring oxygen functional groups. The very low water solubility of phenolic salts of the cannabinoids probably contributes to these observations. Sodium bisulfite was added to minimize oxidation. The petroleum ether layer was separated, washed with water, dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to obtain the nonacidic cannabinoids. The sodium hydroxide extract was acidified with cold 10% sulfuric acid, extracted with ether, washed with water, dried over anhydrous sodium sulfate, and evaporated *in vacuo* at room temperature to obtain the cannabinoid acids.

Table I—Δ^9-Tetrahydrocannabinol and Its Acids in Mexican Strain Marijuana

Percent of Dry Weight[a]	Immature 1969	Female 1969	Female 1968	Male 1969	Male 1968
I	0.22	0.16	0.57	0.45	0.50
II and III	1.00	1.00	0.43	0.71	0.10

[a] Each figure represents the average of two determinations that were within 10% variance.

Table II—Cannabinoids and Cannabinoid Acids in Foreign Strains of Fiber-Type Marijuana

Percent of Dry Weight[a]	Turkish Male	Turkish Female	Swedish	Carmagnola (Italian)	Fibriman (French)	Fibranova (Italian)
I	0.01	0.04	Trace	0.06	Trace	Trace
II and III	0.06	0.02	Trace	0.15	0.05	0.13
IV	0.52	0.44	0.03	0.08	0.08	0.21
V	0.60	0.80	0.04	0.10	0.12	0.45

[a] Each figure represents the average of two determinations that were within 10% variance.

GLC—Both acidic and nonacidic fractions were analyzed using an instrument[1] equipped with flame-ionization detectors and a 0.31-cm. × 3.1-m.(0.125- in. × 10-ft.) stainless steel column packed with 2% OV-17 on 100–120-mesh Gas Chrom Q. Helium was used as carrier gas at 40 ml./min. The oven temperature was kept at 210°, while the inlet and detector temperatures were 250 and 280°, respectively. A steroid internal standard (4-androstene-3,17-dione) was added to each extract. The instruments were calibrated with reference samples of I and IV. The areas under the peaks were directly correlated with the concentration of the compounds (11). The retention time of I was used for the analysis of I and its two respective acids. Similarly, the retention time of IV was used in the analysis of IV and V.

TLC—Each sample was examined by TLC to determine if the separation of acids and nonacids was complete.

Plates—Silica gel GF was coated[2] at a thickness of 0.25 mm. and was activated by warming at 105° for 1 hr.

Solvent Systems—Both acidic and nonacidic fractions were chromatographed in two solvent systems: System A, benzene; and System B, hexane–ether–acetic acid (87:12:1). In System A, the cannabinoids migrated and separated but the cannabinoid acids remained at the starting point. In System B, the acids migrated and separated. Thus, it was possible to tell the presence or absence of the acids as well as the number of acids in the acid fractions. Authentic reference samples of I or IV were always used on each plate.

Detection of Spots—All plates were viewed under a short wavelength UV lamp. All of the cannabinoid acids, as well as IV, quenched the fluorescent background of the plate and were easily detected. After locations of these spots were outlined, the plates were sprayed with freshly prepared diazotized benzedine reagent (12). This reagent yielded a characteristic yellow-orange color with IV, a red-orange color with I, and a brown color with the cannabinoid acids. The R_f values of I in Solvent Systems A and B averaged as follows: A = 0.42 and B = 0.36. The R_x values (x = I) of the other compounds in both systems were: II, A = 0.00 and B = 0.91; III, A = 0.00 and B = 0.86; IV, A = 1.19 and B = 1.11; and V, A = 0.00 and B = 0.64.

RESULTS AND DISCUSSION

Only one strain of the marijuana utilized for this study, the Mexican strain, is of the drug type (high in Δ^9-tetrahydrocannabinol and its acids). Data obtained from the analysis of five samples of this strain are presented in Table I.

The 1969 samples were 6 months old and the 1968 samples were approximately 18 months old at the time of analysis. The lower proportion of acid content in the 1968 samples compared with the 1969 samples suggests that the acids undergo decarboxylation upon storage.

The acidic fraction in System B showed two spots, indicating the presence of both II and III. This was further confirmed by the IR spectra of the acidic fraction and the methyl ester derivative prepared by reaction with diazomethane (2).

[1] Beckman GC-5 or GC-45.
[2] By a Desaga apparatus.

Table III—Cannabinoids and Cannabinoid Acids in Domestic[a] Marijuana

Percent of Dry Weight	Iowa I	Iowa II	Des Moines	Minnesota I	Minnesota II	Minnesota III
I	0.34	Trace	Trace	0.04	0.03	0.08
II and III	0.20	Trace	Trace	Trace	Trace	0.04
IV	0.14	0.38	0.60	0.11	0.07	0.63
V	0.25	0.33	0.33	0.27	0.33	0.11

[a] These samples, with the exception of Minnesota III, were collected in the wild in September 1968.

The other foreign strains of marijuana produced in the drug plant garden in 1969 were of the fiber type (low in Δ^9-tetrahydrocannabinol and high in cannabidiol). The results of the analyses of plants produced in 1969 from these strains are illustrated in Table II.

The cannabinoid acid content of each of these samples was higher than the corresponding cannabinoids. Although each of these marijuana samples was prepared from plants at about the same stage of maturity (flowering stage), the cannabinoid content varied considerably, the lowest being the Swedish strain which is almost devoid of cannabinoids.

Three samples of marijuana collected in Iowa and two samples collected in Minnesota (near Minneapolis) were compared with a sample of marijuana grown on campus in 1969 from seed found in the Minnesota I marijuana (Table III). With the exception of the Iowa I sample, which contained a moderately high quantity of the active I, II, and III, all other domestic varieties investigated contained mainly IV and V and were almost void of the active entities. The change of growing conditions provided to the Minnesota III sample did not change this pattern, *i.e.*, did not trigger the plant to produce active entities in any significant quantities.

SUMMARY AND CONCLUSIONS

A procedure was developed for the assay of the acidic and nonacidic cannabinoids, qualitatively and quantitatively, by chemical fractionation prior to GLC and TLC analyses. Various samples of foreign and domestic, wild and cultivated marijuana were analyzed. From the data obtained, the following were demonstrated.

1. Cannabinoids occur in nature as acids or nonacids with comparable abundance.
2. Tetrahydrocannabinoic acids undergo decarboxylation upon storage or exposure to heat.
3. Plants vary significantly in their cannabinoid composition due to heredity.
4. Change in environment does not change the cannabinoid pattern in plants.

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