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Analysis of Old Samples of *Cannabis sativa* L.

Relatively few phytochemical studies have been conducted on very old samples of *Cannabis sativa* L., particularly leaf and stem material [*1–4*]. At least one article [*5*] has shown that the fluid extract of marihuana is stable for as long as 40 years. A more recent article [*6*] reports on the stability of cannabinoids in stored plant material at different temperatures.

It may be generally stated that some data are available on the type and rate of deterioration of the pure cannabinols, but little is known and reported on the deterioration of cannabinols in relatively old samples of plant material. Because of the obvious legal and forensic aspects of this problem it became of interest to us to study certain old plant samples of *Cannabis sativa* L. which became available on inventorying stored drug samples in our college.

Experimental Methods

The three major old samples of *Cannabis sativa* L. were obtained from stored samples of drugs at the Philadelphia College of Pharmacy and Science. Two of the samples were stored in S. B. Penick Co. cardboard containers and labeled, respectively: "Indian hemp," 1965, and "American Cannabis," 1937. One sample was stored in a ground glass-stoppered glass cylindrical jar and labeled, "Indian Cannabis," with directions to make the tincture and to proceed as directed by the U.S. Pharmacopoeia, 1880. This sample was packaged by Gilpin, Langdon & Co. of Baltimore, Md. On contact with the successors to this company, it was revealed that the sample was indeed as old as the labeled U.S.P. reference date of 1880.

Microscopic analysis revealed that all the samples consisted of mainly leaf material. The ambient temperature of the large metal safe in which the samples were stored probably ranged between 25 and 30°C. All samples were dry and at about a sieve 40 particle size.

Two other samples were also analyzed during this study. They included one sample of *Cannabis* leaf material obtained from the Philadelphia Police Department laboratories and one living plant of approximately 6 months age (24 in. in height). Both the leaf material and the seeds were considered to be of recent Mexican origin by the police. The plant was grown under greenhouse conditions in Philadelphia.

The extraction procedure was based on a slight modification of the procedure reported by Lerner [*1*]. It essentially consisted of extraction of 1 g of plant material in 40 ml of

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chloroform at refrigeration temperatures with shaking at 10-min intervals for 60 min. After filtration, the chloroform was evaporated *in vacuo* at 40°C. The residue was redissolved in 25 ml of ethanol (5 × 5-ml aliquots) and filtered. After reduced pressure evaporation, the residue was redissolved in 1 ml of ethanol containing 1 mg of 4-androstene-3,17-dione as the internal standard. One microlitre of this solution was directly injected in the gas chromatograph. Aliquots of these same solutions were also used for the thin-layer chromatographic analysis.

The gas chromatographic (GLC) analyses were performed on a Hewlett Packard Model 5750 gas chromatograph, equipped with flame ionization detectors and operated isothermally at 230°C with an inlet temperature of 240°C. The column length was 6 ft by ⅛ in. diameter and it was packed with 3 percent OV-17 (phenyl methyl silicone).

Helium was used as the carrier gas at a flow rate of 30 mg/min+ at 53 psi. Peak area measurements were made using the procedure of peak height times width at half-height. Each peak area was compared to the peak area of the internal standard. The GLC of the 1880 sample is given in Fig. 1.

Authentic samples of cannabinol, cannabidiol, and hashish resin were obtained from Dr. K. Genest of the Food and Drug Directorate, Ontario, Canada. The authentic sample of Δ^1 tetrahydrocannabinol (Δ^1THC) was obtained from the Philadelphia Police Department, police laboratories.

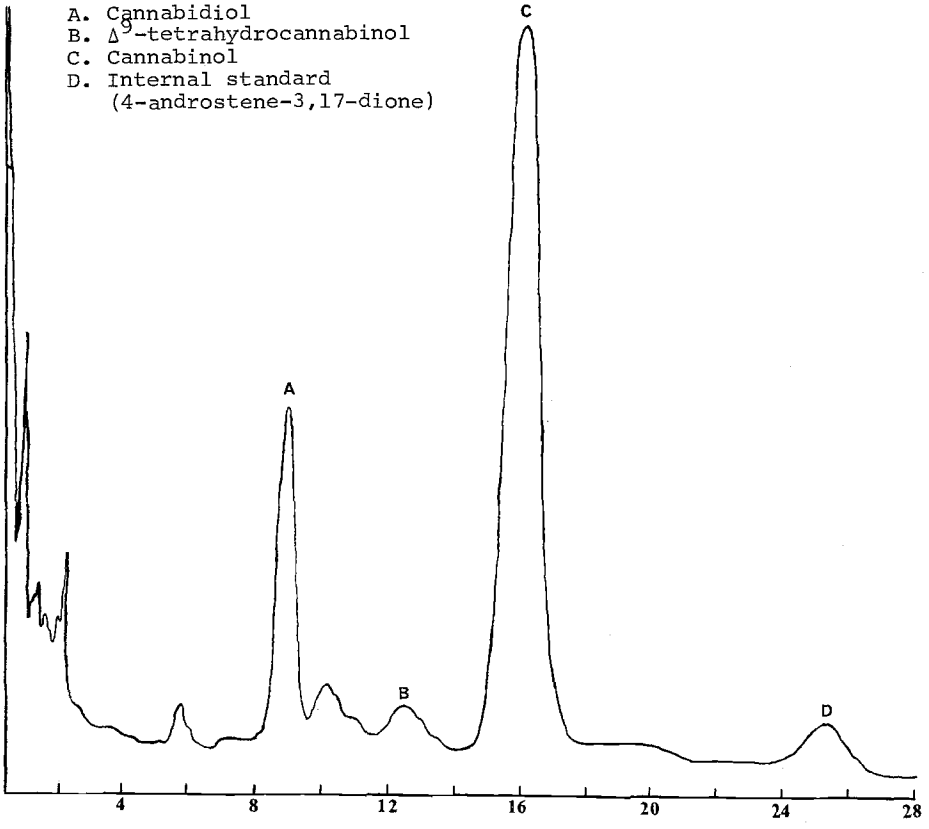

FIG. 1—*Gas chromatographic separation of cannabinoids of Indian* Cannabis *sample (1880).*

The thin-layer chromatography (TLC) was carried out using four different solvent systems with silica gel G as the matrix. The different TLC systems included: petroleum ether:ether (8:2) [7]; Silica gel G soaked in DMF:acetone (1:3) with a solvent system of DMF:cyclohexane (25:150) [8]; Silica gel G impregnated with 20 percent silver nitrate and a solvent system of benzene [9]; and carbon tetrachloride:acetone (98:2) [10]. The TLC runs were monitored using UV light and visualized with Fast blue B (dianisidine tetrazolium Cl), 0.4 percent in alcohol. The R_F values of the major constituents of the different *Cannabis* samples are given in Table 1.

TABLE 1—RF (*multiplied by 100*) *values of the major constituents of different* Cannabis *samples.*

Samples	Cannabidiol				Cannabinol				THC			
Solvent system	I	II	III	IV	I	II	III	IV	I	II	III	IV
Indian *Cannabis*, 1880	92	18	10	50	82	47	65	32	87	67	45	32
Indian *Cannabis*, 1965	91	18	10	50	82	47	66	32	87	66	43	32
American *Cannabis*, 1937	92	18	10	50	[a]	47	70	[a]	[a]	[a]	[a]	[a]
Police sample	92	17	10	45	[a]	47	[a]	[a]	87	68	42	[a]
Fresh leaves	92	[a]	10	[a]	[a]	47	[a]	32	87	65	42	32
Cannabidiol (control)	92	17	10	50	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Cannabinol (control)	[a]	[a]	[a]	[a]	82	47	65	32	[a]	[a]	[a]	[a]
THC (control)	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	87	67	40	32

I = Silicagel-G plate—petroleum ether:ether (8:2).
II = Silicagel-G soaked in DMF and acetone (1:3)—DMF:cyclohexane (25:150).
III = Silicagel-G impregnated with 20 percent $AgNO_3$—benzene.
IV = Silicagel-G—carbon tetrachloride:acetone (98:2).

[a] Compound not observed.

Results and Discussion

Reference to the data on gas-liquid chromatography and thin-layer chromatography reveals that in the majority of samples the three major cannabinoids [namely, cannabidiol, cannabinol, and tetrahydrocannabinol (Δ^1THC)], were readily detected. The only exception was the sample of hashish which was over two years old and which had been kept at room temperature for this period of time. It showed only cannabidiol (using OV-17, this peak actually represents a composite of cannabichromene and cannabidiol) and cannabinol and no THC. This hashish sample did possess THC when it was first received. Apparently, THC is not stable in the hashish resin form when stored at room temperature for long periods of time. This is in line with the early findings of Levine [4] and others when they studied "charas" resin from marihuana. In addition, the *Cannabis* fresh leaf sample (6-month-old plant) showed the presence of cannabidiol and a large amount of THC (1 percent) but no detectable cannabinol by GLC. However, a very small amount of Δ^1THC was visible on thin-layer chromatographic analysis also. The police sample also showed no cannabinol. This sample was of unknown origin, but was considered to be from leaf material about a year old. The American *Cannabis* (1937) likewise showed no cannabinol by GLC and two of the TLC systems. This was considered unusual since cannabinol, a normal end product, would have been considered to be present in this old sample. It has been reported by Ohlsson [11] that cannabinol may not be found in fresh *Cannabis* leaf material and that it may be an artifact. This would account for its lack in the fresh and police samples analyzed.

Perhaps the most surprising result was the detection of significant quantities of THC in the older samples, namely, 0.08 percent in the Indian *Cannabis* (1965), 0.03 percent in American *Cannabis* (1937), and 0.05 percent in the Indian *Cannabis* (1880) sample. As far as we have been able to determine, these findings represent the oldest samples of *Cannabis sativa* which have been found to contain THC. The use of both GLC and TLC confirms the presence of the THC in the plant material as such. Of course, the percentage figures of THC really represent all the potential THC, since some is considered to arise or be formed from the THC acids under the heating conditions of the gas chromatographic analysis. These results seem to contradict the findings of Lerner [*1*] who reported the apparent conversion of all Δ^1THC to cannabinol in a five-year-old sample of marihuana. Perhaps the storage or processing conditions were different for his sample. An old report by Hamilton [*2*] revealed no substantial loss of activity in a sample he studied which was 10 years old.

TABLE 2—*Table showing the percentage content of cannabinoids in different* Cannabis *samples.*

Sample	THC	Cannabidiol	Cannabinol
Police sample	0.03	0.3	...
Indian *Cannabis* (1880)	0.05	0.3	1.7
Indian *Cannabis* (1965)	0.08	0.2	1.2
American *Cannabis* (1937)	0.03	0.05	...
Fresh leaf	1.00	0.08	...
Hashish resin	...	0.12	0.1

The finding of relatively large percentages of cannabinol (for example, Indian *Cannabis* (1880), 1.7 percent; Indian *Cannabis* (1965), 1.2 percent) in two of the old samples is also considered significant, since this is the cannabinoid to which the Δ^1THC and Δ^1THC acids ultimately convert. The apparent lack of cannabinol in the American *Cannabis* (1937) would be due to original low content of Δ^1THC, which is its biogenetic precursor in the plant.

Suffice it to say that in powdered leaf plant material there is some means by which the THC can remain in a fairly stable form under the right conditions. Perhaps the cannabinoids can retain stability as long as they remain contained within the glandular heads of the glandular trichomes of the leaves. Here they are apparently hermetically sealed and thus less prone to oxidation, hydrolysis, and any enzymatic action which may be brought into play during processing or extraction. It is known that pure THC itself deteriorates on the order of 3–5 percent per month at room temperature [*1*]. Most laboratories store THC in a freezer for this reason [*1*]. Turner et al [*6*] report that freezer temperatures (for example, −18, 4, and 22°C) are suitable for normal prevention of decomposition. The postulated conversion or deterioration of the cannabinoids yielded by biosynthetic, synthetic, and extraction studies appears to follow the progression: cannabinolic acid to Δ^1THC or $\Delta^{1(6)}$THC and ultimately to cannabinol. Mechoulam [*12*] has further pointed out that the oily Δ^1THC is labile and is easily isomerized by acids to $\Delta^{1(6)}$THC. Particularly in the presence of air, Δ^1THC is slowly oxidized to cannabinol and probably to compounds of higher molecular weight. These higher molecular weight compounds can be formed via phenolic oxidation. Thus, Δ^1THC considered pure by gas chromatographic analysis may actually contain significant quantities of nonvolatile polymers. These polymers may also show up on thin-layer chromatography and perhaps represent some of the unknowns encountered in our studies. We have attempted here to

concentrate only on the three major constituents for this reason. More work needs to be done on identifying all these closely related deterioration products which do show up on TLC. Suffice it to say that due to a lack of suitable controls the various unknowns encountered in the gas chromatographic studies and thin-layer chromatographic work could not be definitively identified. Undoubtedly they represent various isomeric tetrahydrocannabinol derivatives and possibly cannabicyclol, cannabichromene, cannabigerol, and cannabidiolic acid, as well as possibly hexahydrocannabinol [*6*].

It is unfortunate that analysis of these older samples could not have been undertaken throughout the years to follow the course of changes which occur with the cannabinoids in intact plant material. Nevertheless, the results do indicate a surprising degree of stability of the psychotomimetically active principle (namely, Δ^1THC) over the years, when processed and stored in glass or airtight containers in the absence of light and at room temperature as the powdered leaf and stem preparation. The results of Turner et al [*6*] have indicated, on the other hand, that the Δ^1THC content of *Cannabis sativa* L. stored at -18, 4, and 22 $\pm$ 1°C decomposed at a rate of 3.83, 5.38, and 6.92 percent respectively, per year, whereas the material stored at 37 and 50°C, which they examined, showed considerable decomposition. It is likely that the majority of Δ^1THC in a plant sample will decompose rapidly within a few years, but that some trace amounts will always be detectable even several years later, as shown in this study.

Summary

Analysis of *Cannabis sativa* L. leaf samples ranging in age from 7–90 years of age has revealed the presence of cannabinol, cannabidiol, and tetrahydrocannabinol in varying concentrations. Samples are of American and Indian origin. Analysis methods included both thin-layer and gas chromatography.

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