Cannabis plants illicitly grown in Jutland (Denmark)

E. Kaa

Institute of Forensic Medicine, Department of Forensic Chemistry, University of Aarhus, Skovagervej 2, DK-8240 Risskov, Denmark

Summary. Four hundred forty-nine fresh cannabis plants and 26 fruiting tops harvested in Jutland (Denmark) from July to September 1988 were characterized according to weight, height, marihuana yield, and cannabinoid content. The median weights were 308 g and 584 g for plants grown outdoors (n = 418) and in greenhouses (n = 31), respectively.

The average marihuana yield was 8.7% for the plants grown outdoors and slightly lower for the greenhouse plants. Great variations, however, were seen both between and within the individual harvests. The mean concentration of total THC (tetrahydrocannabinol) was 0.87% for the plants grown outdoors. An increase according to the month of harvest was observed. For plants grown in greenhouses the mean value of total THC was 1.35%, while the mean concentration of fruiting tops was 2.13%. All plants contained cannabidiol (CBD), but only negligible concentrations of other cannabinoids. In approximately 80% of the plants the THC content was higher than the CBD content (drug type), while the rest either contained equal concentrations (intermediate type) or most CBD (fiber type).

Key words: Cannabis plants, harvested in Jutland – Marihuana yield, for plants grown in Jutland – THC

Zusammenfassung. Vierhundertneunundvierzig frische Cannabispflanzen und 26 Fruchtstände, geerntet in Jütland (Dänemark) von Juli bis September 1988, wurden nach Gewicht, Höhe, Mariuhanaertrag und cannabinoidem Inhalt bestimmt. Die Durchschnittsgewichte betrugen 308 g und 584 g in Pflanzen aus Freilandanbau (n = 418) bzw. Gewächshäusern (n = 31). Der durchschnittliche Marihuanaertrag lag bei 8.7% in den Pflanzen aus Freilandanbau und leicht darunter in den Gewächshauspflanzen. Jedoch gab es zwischen den einzelnen Ernten und auch innerhalb dieser große Schwankungen. Die durchschnittliche Totalkonzentration von THC (Tetrahydrocannabinol) betrug bei den Pflanzen aus Freilandanbau 0.87%. Es wurde ein dem Erntemonat entsprechender Zuwachs beobachtet. Für Gewächshauspflanzen lag der durchschnittliche totale THC-Wert bei 1.35%. Die Durchschnittskonzentration in Fruchtständen betrug 2.13%.

Alle Pflanzen enthielten Cannabidiol (CBD), andere Cannabinoide jedoch nur in unwesentlichen Mengen. In annähernd 80% der Pflanzen lag der THC-Inhalt höher als der CBD-Inhalt (Drogentyp). Der Rest enthielt entweder gleich große Konzentrationen (Zwischentyp) oder mehr CBD (Fasertyp).

Schlüsselwörter: Cannabis, Anpflanzungen in Dänemark – Marihuanaertrag, bei Cannabisanpflanzungen – THC, Gehalt bei Freilandanbau in Dänemark

Introduction

The botanical classification of the plant from which marihuana is derived is *Cannabis sativa* L. Δ^9 -THC (tetrahydrocannabinol) is the major psychoactive constituent of the plant. Both the plant and THC are controlled substances and are not allowed in Denmark without special permission from the health authorities. In 1985, the Supreme Court stated that possession of all parts of a cannabis plant except the seeds is illegal. Previously, growing of the plant was allowed when misuse of the euphoric effect was not intended. Naturally, this rather weak formulation caused many misunderstandings, and convictions were difficult to obtain.

Cannabis is the most frequently misused illegal drug in Denmark [1, 16]. Most of the cannabis resin is imported from countries, such as Marocco and Lebanon, whereas most of the marihuana (leaves and flowering/fruiting tops) derives from plants grown in Denmark. In the 1980s the annual amount of cannabis plants seized by the police has varied between 0.4 and 3.4 tons [1]. In 1988, the amount was estimated to be approximately 6–7 tons, mostly having been seized in Jutland [18]. The individual harvests of that year varied between a few plants grown for the owners personal use and large fields (max. 1200 kg) grown for commercial purposes.

The forensic chemical laboratories analyze cannabis plants seized by the police to determine the THC content and the marihuana yield of a harvest. To characterize cannabis illicitly grown in Denmark, this study includes a representative selection of plants seized by the police in Jutland in 1988. The plants were analyzed with regard to weight, height, marihuana yield, and cannabinoid content.

Materials and methods

Plant material

The material consists of 449 fresh, illicit cannabis plants harvested at 22 different locations in Jutland between July 21 and September 27, 1988. Four hundred eighteen of the plants were

grown outdoors and 31 in greenhouses. Of the outdoor plants, 137 were harvested in July, 149 in August, and 132 in September. In addition to entire plants, 26 fruiting tops were analyzed with regard to cannabinoid content.

Approximately 20 plants from each harvest were sent to the forensic chemical laboratory immediately upon seizure. These plants, which were selected by the police, formed a representative sample of the entire harvest. The plants were harvested and weighed without roots. In this study, no discrimination between male and female plants was made, but the vast majority were female.

Chemicals

The reference cannabinoids Δ^9 -THC (tetrahydrocannabinol), Δ^8 -THC (tetrahydrocannabinol), CBD (cannabidiol), CBN (cannabinol), and CBG (cannabigerol) were obtained from Makor Chemicals, Israel. In the following THC refers to Δ^9 -THC.

Preparation procedure

Immediately upon arrival at the laboratory the plants were weighed, and their heights measured. The leaves and flowering/fruiting tops of each plant were picked off and weighed, after which they were laid out on paper and dried at room temperature. After drying, the plant material (marihuana) was weighed again and homogenized prior to the extraction (5.0 g in 100 ml methanol).

To obtain the results of total THC (THC + THCA) and total CBD (CBD + CBDA) all extracts were analyzed by gas chromatography. To differentiate between the neutral and acid form of the cannabinoids, a high-pressure liquid chromatographic analysis was performed on one sample of each harvest [3].

Gas chromatography (GC)

Fifty microliters of C-28 1 μ g/ μ l was added to 200 μ l of the extract as an internal standard. The mixture was carefully evaporated to dryness under a stream of nitrogen at room temperature. The residue was dissolved in 200 μ l n-heptane.

As external standards, 200 μ l THC 0.5 μ g/ μ l and a mixture containing CBD 0.2 μ g/ μ l, CBN 0.2 μ g/ μ l, CBG 0.1 μ g/ μ l, and Δ^8 -THC 0.1 μ g/ μ l were treated in a similar way as the unknown samples.

A Hewlett Packard 5590 gas chromatograph equipped with a flame ionization detector and automatic injection was used. The GC was connected to a HP 3396A integrator. The injection mode was splitless. The column was a 15-m SPB-1 column (Supelco) with an internal diameter of 0.53 μ m and a film thickness of 1.5 μ m. One microliter of the samples was injected.

The instrument settings were as follows: detector temperature, 350°C; injector temperature, 275°C; oven, initial temperature, 60°C; initial time, 1.5 min; temperature program rate, 30°C/min; final temperature, 300°C; final time, 8 min. The retention times of the cannabinoids and the internal standard were: CBD, 11.91; Δ^{8} -THC, 12.33; Δ^{9} -THC, 12.50; CBG, 12.73; CBN, 12.95; C-28, 14.74. All peaks separated well.

Reproducibility

The reproducibility of the extraction was measured using ten samples of a single herbal material (87 g marihuana containing 1.49% total THC). In addition, the reproducibility of the GC preparation (addition of internal standard, evaporation, and dissolution) and the GC injection procedures was measured (n = 10). The coefficient of variation for the total procedure (extraction, GC preparation, and GC injection) was 3.4%. The corresponding figure for the GC preparation and GC injection together was 1.8% while the GC injection procedure, solely, accounted for a coefficient of variation of 1.6%.

	Outdoor	rs(n = 418)	Greenhouse $(n = 31)$		
	Mean	(Range)	Mean	(Range)	
Weight ^a	406 g	(10-2196 g)	921 g	(21–3778 g)	
Height	160 cm	(60-250 cm)	204 cm	(75-330 cm)	
Leaves & flowering/fruiting tops	33%	(11-79%)	28%	(9-51%)	
Yield	8.7%	(2.5-20.2%)	7.5%	(2.1–13.6%)	
Total THC	0.87%	(0.03-4.36%)	1.35%	(0.53-3.26%)	
Total CBD	0.22%	(0.01-2.45%)	0.16%	(0.05-0.68%)	

Table 1. Cannabis grown in Jutland in 1988

^a Median values: 308 g (outdoors), 584 g (greenhouse)

Results

The appearance of the cannabis plants received varied greatly. Some were very bushy, whereas other consisted of only a single stem. Some had leaves all along the stem, when others barely had any leaves but only fruiting tops at the top of the stem. In addition, the maturity of the plants varied according to the date of harvest.

Tables 1 and 2 show the results obtained from examination of all cannabis plants taken together and the individual harvests, respectively. Table 1 also includes cannabis grown in greenhouses (31 plants from four harvests). Both height and weight of greenhouse plants were higher as compared to plants grown outdoors.

When fresh, the leaves and flowering/fruiting tops accounted for one third of the weight of the plant on average. Especially small plants had a high percentage (nos. 155 and 171, Table 2). The mean weight loss upon drying was 74% (range: 68%-77%).

The marihuana yield is the proportion which dried leaves and flowering/ fruiting tops account for as compared to the weight of the entire plant (fresh). The mean yield of marihuana by weight was 8.7% (SD 3.3%) for the 418 plants grown outdoors. The greenhouse plants had a slightly lower yield (Table 1). For the 22 outdoor harvests the range was 6.1%-17.3% (Table 2). The yield increased according to the month of harvest (Table 3).

The mean value of total THC for all 418 plants grown outdoors was 0.87% (SD 0.55%), but great variations within the individual harvests were seen (Table 2). The content of THC increased according to the month of harvest (Table 3). In July, only 15% of the plants had a THC content or more than 1.0% as compared to 26% in August and 55% in September. The cannabinoids occurred in the plants principally as their acids. The mean ratio THCA/THC was 4.0 (SD 3.9).

The mean THC contents of plants grown in greenhouses was significantly higher than those of plants grown outdoors (Table 1). The contents of total THC in fruiting tops were even higher (Table 4).

Table 2.	Table 2. Cannabis grown outdoors in Jutland. Mean values of 22 harvests	outdoors in Juti	land. Mean	values of 22	harvests						
No.	Date of harvest	Number of plants	Height (cm)	Weight (g)	Leaves & flowering/ fruiting tops	Yield (%)		Total CBD (%)	Total THC (%)		CBD/THC
					(%)	Mean	(SD)		Mean	(SD)	
118	21/07/88	18	94	63	32	9.4	(3.1)	0.19	1.05	(0.21)	0.2
127	27/07/88	21	182	690	33	7.9	(1.3)	0.19	0.37	(0.24)	1.6
128	27/07/88	19	212	566	25	6.4	(1.2)	0.10	0.55	(0.18)	0.2
129	29/07/88	19	126	188	37	9.9	(2.2)	0.12	0.51	(0.16)	0.3
130	29/07/88	20	178	292	28	7.3	(1.7)	0.27	0.66	(0.26)	0.5
131	29/07/88	20	179	368	30	7.4	(1.5)	0.08	0.67	(0.21)	0.1
132	29/07/88	20	165	391	24	6.2	(1.4)	0.14	0.93	(0.16)	0.2
133	04/08/88	20	156	1021	25	6.3	(1.2)	0.14	0.59	(0.22)	0.3
135	05/08/88	20	188	474	26	6.1	(1.2)	0.06	0.61	(0.18)	0.1
136	05/08/88	20	164	213	32	7.3	(1.3)	0.10	0.57	(0.23)	0.3
138	03/08/88	20	142	335	37	9.7	(3.2)	0.10	0.95	(0.20)	0.1
144	12/08/88	14	207	920	33	8.3	(1.2)	0.08	1.15	(60.0)	0.1
148	22/08/88	20	181	522	30	7.9	(1.2)	0.19	0.71	(0.27)	0.4
149	25/08/88	19	127	298	39	11.3	(2.1)	0.72	0.82	(0.79)	2.8
150	25/08/88	16	158	232	40	11.6	(2.2)	0.48	0.89	(0.72)	1.9
153	05/09/88	20	203	718	35	8.7	(3.2)	0.25	0.68	(0.37)	0.8
154	05/09/88	18	148	356	27	6.5	(1.8)	0.28	0.84	(0.47)	0.9
155	05/09/88	19	141	151	49	14.1	(2.4)	0.25	0.97	(0.43)	0.4
157	88/60/90	20	122	296	31	7.0	(2.0)	0.12	1.29	(0.41)	0.1
158	06/09/88	20	183	325	28	7.4	(1.8)	0.23	0.95	(0.39)	0.3
166	15/09/88	20	129	367	38	10.5	(2.6)	0.61	2.02	(0.95)	0.5
171	27/09/88	15	123	129	56	17.3	(1.6)	0.18	1.60	(0.57)	0.2
Total:		418	160	406	33	8.7	(3.3)	0.22	0.87	(0.55)	0.6

Cannabis plants

	July (<i>n</i> = 137)		Augus $(n = 14)$		September $(n = 132)$		
	Mean	(Range)	Mean	(Range)	Mean	(Range)	
Weight ^a	372 g	(23–1525 g)	494 g	(10–1787 g)	343 g	(13-2196 g)	
Leaves & flower-							
ing/fruiting tops	30%	(12-58%)	33%	(11-58%)	37%	(14-79%)	
Yield	7.8%	(3.0-15.6%)	8.5%	(2.5 - 18.5%)	10.0%	(3.4 - 20.2%)	
Total THC	0.67%	(0.03-1.47%)	0.77%	(0.05-2.94%)	1.18%	(0.07-4.36%)	
Total CBD	0.15%	(0.01-0.64%)	0.23%	(0.01-2.45%)	0.28%	(0.02 - 1.53%)	
CBD/THC	0.5	(0-9.7)	0.7	(0-15.3)	0.4	(0-9.1)	

Table 3. Cannabis grown outdoors. Mean values according to the month of harvest

^a Median values: 306 g (July), 378 g (August), and 220 g (September)

 Table 4. Contents of cannabinoids in fruiting tops obtained from seven harvests at different locations

Date of harvest	Number	Total THC (%)		Total CBD (%)		CBD/THC	
		Mean	(Range)	Mean	(Range)	Mean	(Range)
21/07	1	1.52		0.13		0.1	
07/09	1	1.00		0.14		0.1	
22/09	7	1.60	(0.70 - 2.75)	0.37	(0.11-0.93)	0.3	(0.1 - 1.3)
23/09	1	3.10		0.27		0.1	
26/09	10	3.02	(1.17 - 4.89)	0.10	(0.04 - 0.23)	0.0	(0.0 - 0.1)
27/09	5	1.19	(0.26 - 3.34)	0.44	(0.10 - 1.05)	0.1	(0.1 - 4.0)
27/09	1	2.57	. ,	0.24		0.1	. ,
Total:	26	2.13	(0.26-4.89)	0.25	(0.04-1.05)	0.3	(0.0-4.0)

All plants contained cannabidiol (CBD), while the contents of other cannabinoids (Δ^8 -THC, CBN, and CBG) were low. A small increase in contents of CBD during the season can be noticed (Table 3).

Seventy-nine per cent of the plants contained more THC than CBD (CBD/THC < 0.75). Thirteen per cent contained approximately the same amount of the two cannabinoids (0.75 < CBD/THC < 1.25), while the CBD content in 8% of the plants was higher than the THC content (CBD/THC > 1.25). In three of the harvests most of the plants contained more CBD than THC (nos. 127, 149, and 150, Table 2), but even within the individual harvests great variations were found.

Discussion

Most studies on cannabis plants have been done on plants grown legally for research purposes [2, 5, 7, 8, 19]. In this way, it has been possible to use seeds of known origin to study species and sex specifications. While previously only the female plant was claimed to contain psychoactive cannabinoids, the male plant is now also considered to contain active constituents [20]. Fruiting tops have been found to have the highest content of THC, the stem only a low content, and the seeds none [15, 20]. Marihuana obtained from plants consisting mainly of fruiting tops with the lower leaves stripped off is therefore expected to have a higher THC content than plants still having their lower leaves. A special sort of marihuana – sinsemilla – is the seedless material that results when the female flowers remain unpollinated. Sinsemilla may contain even higher concentrations of THC and is especially common in USA. In 1985 and 1986, one third of the cultivated cannabis eradicated in USA was sinsemilla marihuana [17].

In fresh plants cannabinol (CBN) is normally absent [20], as was the case in this study. All plants, on the other hand, contained CBD. The CBD/THC ratio has been used by many authors to differentiate between plants of the drug type and plants of the fiber type in which THC and CBD, respectively, dominate [9, 13]. Occasionally, an intermediate type containing approximately equal concentrations of the two constituents is also described [22, 24]. In this study, most of the harvests contained plants of all three types and although the drug type, as intended, was the most frequently found, three of the harvests, in fact, mostly consisted of plants of the other two types. The proportion of drug type plants in this study (approximately 80%) corresponds to the results of an Italian study on 114 plants [9].

The literature shows no general agreement as to the stability of the CBD/ THC ratio during the season. Some claim that the ratio does not change during the growing period, while others claim the opposite [8, 10, 21, 24, 25]. The difference may partly be due to whether parts of the plant or entire plants were examined. In this study, great variations in the ratio both between and within the individual harvests were observed. The contents of total THC increased according to the month of harvest, and a minor increase in contents of total CBD may also have taken place. The mean value of the ratio did not change significantly, however, during the 3-month period.

The considerable variations in cannabinoid patterns of plants within the individual harvests indicate that the THC content is dependent on genetic rather than environmental characteristics. This agrees with most other studies [5, 7, 10, 19, 20]. The variation in the ratio may be caused by the use of seeds of different origin. The difference may also be due to variations between first and second generation plants [7].

The GC method used for quantitative determination gives the total THC and total CBD values. Due to heating of the samples in the injection part of the gas chromatograph, this technique converts the acid cannabinoids to the neutral forms [14, 20]. Heating, storage, and influence of light cause the decarboxylation [11, 23]. As heating is normally involved in the illegal use of cannabis (normally smoking), decarboxylation will take place. The total concentration (THCA + THC), as determined by GC, is therefore the best estimate of the psychoactive effect of the product.

As compared with most other studies [4, 6, 12, 25], the mean concentration of total THC in this study was rather low. The difference may in part be due to

whether entire plants or only fruiting tops had been used [12]. A study on second generation plants in the United Kingdom resulted in a mean value of total THC of 0.9% as compared to a concentration of 2.2% the year before [7]. Concentrations of total THC within the range of the results of this study were also seen in an Italian study of drug type plants [2].

The relatively low mean value of total THC in this study is mainly thought to be due to very early harvesting dates, as many seizures were carried out even before the plants had matured. In plants harvested in the last part of September, concentrations of total THC of up to 4.36% and 4.89% in marihuana from an entire plant and a fruiting top, respectively, were found. The mean concentration of total THC in fruiting tops found in this study was higher but within the range of the results of a previous Danish study [12].

References

- 1. Annual Report of the Police (1987)
- Avico U, Pacifici R, Zuccaro P (1985) Variations of tetrahydrocannabinol content in cannabis plants to distinguish the fibre-type from drug-type plants. Bull Narc 37(4):61–65
- 3. Baker PB, Fowler R, Bagon KR, Gough TA (1980) Determination of the distribution of cannabinoids in cannabis resin using high performance liquid chromatography. J Anal Toxicol 4:145-152
- Baker PB, Bagon KR, Gough TA (1980) Variation in the THC content in illicitly imported Cannabis products. Bull Narc 32(4):47–54
- 5. Baker PB, Gough TA, Taylor BJ (1982) The physical and chemical features of cannabis plants grown in United Kingdom of Great Britain and Northern Ireland from seeds of known origin. Bull Narc 34(1):27-36
- Baker PB, Gough TA, Johncock SIM, Taylor BJ, Wyles LT (1982) Variation in THC content in illicitly imported cannabis products – part II. Bull Narc 34(3-4):101–108
- Baker PB, Gough TA, Taylor BJ (1983) The physical and chemical features of cannabis plants grown in the United Kingdom of Great Britain and Northern Ireland from seeds of known origin - part II: Second generation studies. Bull Narc 35(1):51-62
- 8. Barni-Comparini I, Ferri S, Centini F (1984) Cannabinoid level in the leaves as a tool for the early discrimination of cannabis chemiovariants. Forens Sci Int 24:37-42
- 9. Bertol E, Mari F (1980) Observations on cannabinoid content in *Cannabis sativa* L. grown in Tuscany, Italy. Bull Narc 32(4):55-60
- Fairbairn JW, Liebmann JA (1974) The cannabinoid content of *Cannabis sativa* L. grown in England. J Pharm Pharmacol 26:413–419
- 11. Fairbairn JW, Liebmann JA, Rowan MG (1976) The stability of cannabis and its preparations on storage. J Pharm Pharmacol 28:1-7
- 12. Felby S, Nielsen E (1985) Cannabinoid content of cannabis grown on the Danish island of Bornholm. Bull Narc 37(4):87–94
- Fetterman PS, Keith ES, Waller CW, Guerrero O, Doorenbos NJ, Quimby MW (1971) Mississippi-grown *Cannabis sativa* L.: Preliminary observation on chemical definition of phenotype and variations in tetrahydrocannabinol content versus age, sex, and plant part. J Pharm Sci 60:1246–1249
- Fetterman PS, Doorenbos NJ, Keith ES, Quimby MW (1971) A simple gas liquid chromatography procedure for determination of cannabinoidic acids in *Cannabis sativa* L. Experimentia 27:988–990
- Hemphill JK, Turner JC, Mahlberg PG (1980) Cannabinoid content of individual plant organs from different geographical strains of *Cannabis sativa* L. J Nat Prod 43:112–122
- 16. Kaa E (1988) Drug abuse in Denmark (Jutland and Funen) a forensic study based upon drugs seized in 1982–1987. Z Rechtsmed 101:229–236

- National Narcotic Intelligence Consumers Committee (1987) The NNICC Report 1985– 1986
- 18. National Drug Intelligence Unit (private communication)
- Nielsen E (1970) Thin-layer chromatographic analysis of cannabis from Danish and other sources. Dansk Tidskr Farm 44:359–364
- 20. Ohlsson A, Abou-Chaar CI, Agurell S, Nilsson IM, Olafsson K, Sandberg F (1971) Cannabinoid constituents of male and female *Cannabis sativa*. Bull Narc 23(1):29–32
- Philips R, Turk R, Manno J, Jain N, Forney R (1970) Seasonal variation in cannabinolic content of indiana marihuana. J Forens Sci 15:191–200
- 22. Small E, Beckstead HD (1973) Cannabinoid phenotypes in Cannabis sativa. Nature 245:147-148
- Turner CE, Hadley KW, Fetterman PS, Doorenbos NJ, Quimby MW, Waller C (1973) Constituents of *Cannabis sativa* L. IV: Stability of cannabinoids in stored plant material. J Pharm Sci 62:1601–1605
- 24. Turner CE (1980) Marijuana research and problems: an overview. Pharm Int 1:93-96
- 25. Turner CE, Elsohly HN, Lewis GS, Lopez-Santibanez I, Carranza J (1982) Constituents of *Cannabis sativa* L. XX: The cannabinoid content of Mexican variants grown in Mexico and in Mississippi, United States of America. Bull Narc 34(1):45–59

Received December 21, 1988