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## Cannabinoid content of cannabis grown on the Danish island of Bornholm

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### ABSTRACT

The analysis in 1983 of representative samples taken from fruiting tops of seized cannabis plants illicitly grown in 19 localities on the Danish island of Bornholm showed that the average (mean) content of the total tetrahydrocannabinol ( $\Delta^9$ -tetrahydrocannabinol (THC) +  $\Delta^9$ -tetrahydrocannabinolic acid) was approximately 1.55 per cent, ranging from 0.1 to 4.2 per cent. There was no significant difference in the total THC content of cannabis plants from the Danish island of Bornholm and the content found by other authors in cannabis plants grown during the period from 1968 to 1972 in Afghanistan, India, Mexico, South Africa and Thailand. However, studies carried out on fresh cannabis seized on entry into the United Kingdom of Great Britain and Northern Ireland during the period 1975–1981 showed a larger content of THC (ranging from 2.3 to 4.9 per cent) than in cannabis plants from Bornholm.

### Introduction

In the autumn of 1983 the police seized 1.5 tonnes of cannabis\* plants illicitly grown in 19 localities on the Danish island of Bornholm.\*\* In addition, the police found manufactured cannabis (marijuana) in nine localities. In 11 localities pipe ashes containing cannabinoids were found, which showed that the cultivated cannabis plants were abused by the population of the island.

### Material

Four fruiting tops from each of the 19 localities, one top from a large plant, one top from a small plant and two tops from medium-size plants, were sent for analysis. Only four of the tops were staminate or vegetative

\* The term cannabis in this article refers to *Cannabis sativa* L.

\*\* Bornholm is situated in the Baltic, 55° northern latitude and 15° eastern longitude.

plants, while the others were pistillate plants. The plants were separated from woody stems and stalks. The yield of leaves and fruiting parts was 81.5 per cent (standard deviation 6.4 per cent). The leaves and fruiting parts from each individual plant were air-dried and crushed before extraction.

### Method

Chemicals: acetone p.a., n-hexane p.a. and pyridine p.a., all from Merck; trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS), both from Pierce;  $\Delta^9$ -tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN), all from Supelco; and dotriacontane p.a. from Fluka.

Standard: a mixture of 0.67 mg THC, 0.17 mg CBD and 0.17 mg CBN per ml of methanol. Internal standard: dotriacontane 250 mg in 1,000 ml n-hexane.

Extraction: 5.0 g of crushed material from each top was extracted with 100 ml chloroform. After extraction was added anhydrous sodium sulphate, shaken and filtered. Of the filtered extract, 500  $\mu$ l was evaporated in a 10 ml tube in a stream of nitrogen. To the residue was added 500  $\mu$ l of acetone and 1,000  $\mu$ l of internal standard. Standard: 150  $\mu$ l standard was treated as the sample extract. To the residue after evaporation was added 150  $\mu$ l of acetone and 300  $\mu$ l of internal standard.

### Silylation

A 1,000  $\mu$ l sample extract was evaporated as above. To the residue was added 200  $\mu$ l of acetone, 50  $\mu$ l of silylating reagent (1 per cent TMCS in HMDS), 50  $\mu$ l of pyridine and the mixture was left to stand for one hour. After that, 1,000  $\mu$ l of internal standard was added. Standard: 150  $\mu$ l standard was treated as above; 100  $\mu$ l of acetone, 50  $\mu$ l of silylating reagent, 50  $\mu$ l of pyridine and after one hour 300  $\mu$ l of internal standard were added.

### Gas chromatography

The following experimental equipment and procedures were used: gas chromatograph Perkin-Elmer model 3920B equipped with Perkin-Elmer auto-sampler model AS 41 and a Perkin-Elmer data station Sigma-10; the glass columns (2 m  $\times$  2 mm ID) packed with 3 per cent OV-17 on Chromosorb W-AW-DMCS-80-100 mesh; injector 250°C; the flame ionization detector 300°C; carrier gas helium flow 40 ml/min; oven temperature programmed operation with initial temperature 250°C, initial time 1 min, rate 8°C per min, final temperature 290°C, final time 2 min.

## Results

Table 1 shows the cannabinoid content of the 76 fruiting tops of cannabis plants. Free THC +  $\Delta^9$ -tetrahydrocannabinolic acid (THCA) is expressed as the total THC. THCA is decarboxylated to THC by gas-liquid chromatographic analysis (if the acid group is not protected for instance by silylation).

**Table 1**  
**Contents of cannabinoids in 76 fruiting tops from cannabis plants**  
**grown in 19 localities on the Danish island of Bornholm**

Locality code	Plant size	CBD per cent	CBN per cent	Total THC per cent	THC per cent	Ratio of THCA to THC
1	Small	0.1	0.0	1.5	0.3	4.4
	Medium	1.4	0.0	1.0	0.3	2.5
	Medium	<0.1	0.0	1.0	0.2	4.4
	Large	0.0	0.0	1.4	0.1	14.2
2	Small	0.1	0.0	0.9	0.1	8.7
	Medium	0.2	0.1	4.2	0.6	6.5
	Medium	<0.1	0.1	1.9	0.1	19.6
	Large	1.3	0.0	0.3	<0.1	—
3	Small	<0.1	0.0	0.8	0.2	3.3
	Medium	<0.1	<0.1	1.8	0.5	2.8
	Medium	0.1	<0.1	1.5	0.7	1.2
	Large	0.0	0.0	1.3	0.1	13.1
4	Small	0.2	0.1	2.5	2.0	0.3
	Medium	0.1	0.0	2.1	0.5	3.5
	Medium	0.9	0.0	0.7	0.2	2.7
	Large	0.1	0.0	2.3	0.6	3.1
5	Small	0.1	0.1	2.6	0.3	8.4
	Medium	<0.1	0.0	1.4	0.2	6.5
	Medium	0.1	<0.1	1.0	0.1	9.8
	Large	0.1	<0.1	1.9	0.3	5.8
6	Small	0.1	0.0	0.9	0.1	8.7
	Medium	0.1	0.0	1.7	0.3	5.1
	Medium	<0.1	0.0	0.7	0.2	2.7
	Large	1.7	0.0	0.7	0.4	0.8
7	Small	0.1	0.0	1.8	0.1	18.5
	Medium	0.1	<0.1	1.9	0.2	9.3
	Medium	0.1	0.0	1.1	0.1	10.9
	Large	0.1	0.0	2.0	0.1	20.7
8	Small	1.6	0.0	0.1	<0.1	—
	Medium	0.1	0.0	0.9	0.4	1.4
	Medium	1.2	0.0	0.5	0.2	1.6
	Large	0.2	0.0	1.1	0.5	1.3
9	Small	0.1	0.0	1.3	0.2	6.0
	Medium	0.2	0.0	1.5	0.4	3.0
	Medium	0.2	0.1	2.7	0.6	3.8
	Large	0.1	0.1	2.4	0.2	12.0

Table 1 (continued)

Locality code	Plant size	CBD per cent	CBN per cent	Total THC per cent	THC per cent	Ratio of THCA to THC
10	Small	0.1	0.0	1.2	0.1	12.0
	Medium	0.3	0.0	1.4	0.4	2.7
	Medium	0.1	0.0	2.2	0.2	10.9
	Large	0.1	0.0	1.7	0.5	2.6
11	Small	0.2	0.1	2.9	0.1	30.5
	Medium	0.2	0.1	3.0	0.1	31.6
	Medium	<0.1	0.0	0.9	0.2	3.8
	Large	0.1	0.1	1.8	0.3	5.5
12	Small	0.3	0.0	2.0	0.2	9.8
	Medium	0.4	0.0	0.4	0.1	3.3
	Medium	<0.1	0.0	1.0	0.1	9.8
	Large	0.1	0.0	2.1	0.2	10.4
13	Small	0.1	0.0	1.8	0.5	2.8
	Medium	0.1	0.0	1.1	0.6	0.9
	Medium	0.0	0.0	1.1	0.6	0.9
	Large	<0.1	0.0	1.5	0.1	15.3
14	Small	2.6	<0.1	2.3	0.4	5.2
	Medium	0.1	<0.1	2.9	1.1	1.8
	Medium	0.2	0.0	2.3	0.6	3.1
	Large	0.7	0.0	0.8	0.3	1.8
15	Small	1.2	0.1	2.1	0.9	1.5
	Medium	1.3	0.0	0.1	0.1	0.0
	Medium	0.1	0.0	2.3	0.1	24.0
	Large	0.1	0.0	1.0	0.3	2.5
16	Small	<0.1	0.0	1.5	0.4	3.0
	Medium	<0.1	0.1	2.7	0.7	3.1
	Medium	0.2	0.0	2.7	0.3	8.7
	Large	0.1	0.1	3.2	0.3	10.5
17	Small	2.0	0.0	0.6	0.1	5.5
	Medium	0.3	0.0	0.7	0.2	2.7
	Medium	4.5	0.0	0.1	<0.1	—
	Large	0.1	<0.1	2.3	0.6	3.1
18	Small	0.2	<0.1	1.2	0.2	5.5
	Medium	0.7	0.0	0.8	0.4	1.1
	Medium	0.7	0.0	1.2	0.3	3.3
	Large	0.0	0.0	1.0	0.1	9.8
19	Small	0.2	0.0	1.6	0.6	1.8
	Medium	0.2	<0.1	1.3	0.5	1.7
	Medium	0.2	0.0	1.2	0.5	1.5
	Large	0.1	0.0	2.2	0.3	6.9

Table 2 shows that the total THC contents ranged from 0.1 per cent to 4.2 per cent, with the mean 1.55 per cent (standard deviation 0.81). The median of the total THC contents was 1.45 per cent. The content of CBD was between zero and 2.6 per cent and the content of CBN was very low, between zero and 0.1 per cent.

**Table 2**  
**Total THC content of fruiting tops from cannabis plants**  
**grown on Bornholm**

<i>Average</i>	<i>Standard deviation</i>	<i>95 per cent confidence limits (percentage)</i>	<i>Range (percentage)</i>
Mean	0.81	1.37–1.73	0.1–4.2
Median	—	1.20–1.80	

To estimate if the collection method could have some influence on the average total THC content, both one-way analysis of variance and a Kruskal-Wallis non-parametric test were applied to the results of different groups of plants. The results of the tests, tabulated in table 3, show that the total THC content is independent of the collection method used for this research. A quantile diagram of the 76 results of the total THC content and a test of kurtosis (3.28) and of skewness (0.54) showed departure from the normal distribution. The median of the total THC contents (1.45 per cent) is, therefore, a better measure of the middle of the distribution than is the mean (see table 3). For one-way analysis of variance, the sampling distribution is the F-distribution with 2 and 73 degrees of freedom, giving the P-value 0.15 ( $F=0.167$ ). For the Kruskal-Wallis test, the sampling distribution is the chi-square distribution with 2 degrees of freedom, giving the P-value 0.34 ( $\text{Chi}^2=0.835$ ).

**Table 3**  
**Total THC content of fruiting tops from the three groups**  
**of cannabis plants, classified by size**

<i>Size of plants</i>	<i>Number of plants</i>	<i>Total THC content (percentage)</i>		
		<i>Range</i>	<i>Mean</i>	<i>Median</i>
Small	19	0.1–2.9	1.56	1.50
Medium	38	0.1–4.2	1.50	1.25
Large	19	0.3–3.2	1.63	1.70

To examine if there was any difference in the THC contents of the plants from different localities, a goodness-of-fit test was applied, which showed that the observed frequencies followed the binomial distribution, and therefore that there was no significant difference in the total THC content of the plants from different localities (see table 4). For the goodness-of-fit test, the sampling distribution is the chi-square distribution with 3 degrees of freedom, giving the P-value 0.28 ( $\text{Chi}^2=1.35$ ).

**Table 4**  
**Chi-square of goodness-of-fit test of the binomial distribution**

<i>Number of plants<sup>a</sup></i>	<i>Observed frequency</i>	<i>Expected frequency</i>
0	2	1.19
1	3	4.75
2	8	7.13
3	5	4.75
4	1	1.19

<sup>a</sup> Number of fruiting tops from plants with the total THC content higher than 1.45 per cent (the median for 19 localities).

### Discussion

The results of the total THC content that the authors obtained in this research are in agreement with the total THC contents found by Holley and others [1] in cannabis plants grown in Afghanistan, India, Mexico, South Africa and Thailand during the period from 1968 to 1972. The mean of the free THC content reported by the present authors is 0.3 per cent with a standard deviation of 0.3 ranging from 0.1 to 2.0 per cent. This finding is in agreement with the results of previous research carried out by Schou and Nielsen [5] on plants grown in Denmark from seeds provided by the Narcotics Laboratory Section of the Division of Narcotic Drugs, in which the mean of the free THC was found to be 0.5 per cent with a standard deviation of 0.5 per cent ranging from 0.1 to 1.7 per cent.

**Table 5**  
**Comparison of the total THC content of cannabis plants grown in 1983 on the Danish island of Bornholm with the total THC content of cannabis plants grown during the period 1968–1972 in five other countries**

<i>Country</i>	<i>Number of plants</i>	<i>The total THC content (percentage)</i>		
		<i>Range</i>	<i>Mean</i>	<i>Median</i>
Afghanistan	6	0.6–4.4	2.45	2.45
Denmark (Bornholm)	76	0.1–4.2	1.55	1.45
India	15	0.1–4.3	1.86	1.7
Mexico	13	0.1–3.0	1.67	2.0
South Africa	10	0.3–6.1	2.03	1.7
Thailand	8	0.3–2.9	1.5	1.5

Table 5 compares the total THC content obtained from Bornholm's cannabis plants with the results obtained by other authors [1], using both a one-way analysis of variance and a Kruskal-Wallis test. For one-way analysis of variance, the sampling distribution is the F-distribution with 4 and 22 degrees of freedom, giving the P-value 0.87 ( $F=1.80$ ). For the Kruskal-Wallis test, the sampling distribution is the chi-square distribution with 5 degrees of freedom, giving the P-value 0.50 ( $\text{Chi}^2=4.36$ ). These tests showed no significant differences in the total THC contents of cannabis plants from the Danish island of Bornholm and cannabis plants from the countries mentioned above. The work of Baker and others [2] showed that fresh cannabis, seized on entry into the United Kingdom of Great Britain and Northern Ireland over the period 1979--1981, had a larger total THC content (mean 2.3 to 4.9 per cent) than cannabis from Bornholm and that there was a general increase in the total THC content during the period.

Baker and others [3, 4] divided cannabis plants grown in 1980 and 1981 into the following three categories on the basis of CBD to THC ratios: those where the ratio exceeded 10; those where the ratio was approximately unity and those where the ratio was zero for examination of variation in the plant population. Most of the plants that the present authors examined had ratio zero or nearly zero, but the origin of the seeds was not known.

Baker and others [2-4] compared the ratio of THCA to THC in fresh cannabis illicitly imported into the United Kingdom and in harvested material from generation studies in 1980 and 1981.

The present authors have calculated the ratio of THCA to THC for the material used in their research and the results are shown in table 6, together with the results obtained by Baker and others [2-4]. The THCA to THC ratio of the plants investigated by the present authors are of similar order to that obtained for the second generation plants grown in the United Kingdom. Three of the fruiting tops of plants from Bornholm containing only traces of THC were not included in the calculation of the THCA-to-THC ratio.

*Table 6*

**Comparison of the ratio of THCA to THC in cannabis plants from the Danish island of Bornholm with results obtained in the United Kingdom [2-4]**

<i>Plant group</i>	<i>Number of plants</i>	<i>Ratio of THCA to THC (mean)</i>	<i>Standard deviation</i>
Fresh cannabis illicitly imported into the United Kingdom in 1979	64	2.4	2.2
Plants grown in the United Kingdom in 1980	22	4.0	4.6
Plants grown in the United Kingdom in 1981	30	9.4	7.4
Plants grown in Bornholm in 1983	73	6.6	6.6

### Conclusion

The study shows that cannabis plants grown on the Danish island of Bornholm have a relatively high percentage of total THC and that the fruiting tops from small, medium and large plants have on average (median) similar contents of total THC. The ratio of THCA to THC indicates that the cannabis plants from Bornholm were second generation plants.

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