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The main cannabinoids content in hashish samples seized in Israel and Czech Republic

Lumír O. Hanuš[a]*, Rina Levy[b], Dafna De La Vega[b], Limor Katz[b], Michael Roman[c] and Pavel Tomíček[c]

[a]*Department of Medicinal Chemistry and Natural Products, School of Pharmacy, Medical Faculty, The Hebrew University of Jerusalem, Jerusalem, Israel;* [b]*Analytical Chemistry Laboratory, Division of Identification and Forensic Sciences, National Police Headquarters, Jerusalem, Israel;* [c]*Department of Chemistry and Physics, Institute of Criminalistics, Police of the Czech Republic, Prague, Czech Republic*

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Cannabis, both herbal and resin, has been the most popular illicit drug in Israel in recent years. Until 2005, the main sources of cannabis resin (known also as hashish) to the Israeli drug market were Lebanon and India. Hashish from these sources can be distinguished by its external appearance. The aim of this study was to determine if there is any difference in the quality of the hashish from each source. For this purpose, we quantified the main cannabinoids, cannabidiol (CBD), Δ^9-tetrahydrocannabinol (Δ^9-THC), and cannabinol (CBN) of hashish from different police seizures of known origins, Lebanon, India and Morocco, that had been submitted to the Analytical Chemistry Laboratory of the Division of Identification and Forensic Science at the Israeli National Police Headquarters and subsequently to the Hebrew University for quantitative analysis. The results, based on many different seizures, showed that the average CBD content of hashish from Lebanon varied from 5.69% to 12.79% (an average 8.98 ± 0.59%), THC of hashish from Lebanon varied from 0.93% to 4.20% (an average of 2.38 ± 0.27 %), CBD of hashish from Morocco varied from 1.52% to 5.14% (an average of 3.72 ± 0.19%), THC of hashish from Morocco varied from 5.08% to 13.41% (an average of 9.21 ± 0.40%), CBD of hashish from India varied from 0.78% to 13.13% (an average of 4.59 ± 1.07%), and THC of hashish from India varied from 0.53% to 16.45% (an average of 6.35 ± 1.50%), At the same time, several other cannabinoids present in the samples in lower amounts were identified (cannabidivarol, CBDV; cannabicitran; Δ^9-tetrahydrocannabivarol, Δ^9-THCV; cannabivarol, CBV; cannabicyclol, CBL; cannabichromene, CBC; cannabielsoin, CBE; Δ^8-THC; and cannabigerol, CBG). The samples, predominantly from Lebanon, Morocco, and India, were evaluated for chemical phenotype (drug type and fiber type) to determine the geographical origin of these samples.

Indian hashish, seized by Czech Republic authorities, was analyzed qualitatively for many cannabinoids and terpenes. In this sample three new cannabinoids were identified: cannabiorcochromene, *cis*-Δ^9-tetrahydrocannabivarol, and cannabinerol.

Keywords: cannabis resin; hashish; CBD; THC; drug profiling

Cannabis, both herbal and resin, is the most popular illicit drug in Israel and accounts for about 70% of all drug seizures analyzed in the Analytical Chemistry Laboratory of the Division of Identification and Forensic Science at the Israeli National Police Headquarters from 1995 to 2005. In recent years, cannabis resin, known also as hashish, has become more popular among Israeli drug users, who prefer it over herbal cannabis and seek new sources of supply. Most of the hashish enters Israel from Lebanon, while in recent years India and Morocco have become a popular source of hashish for Israeli drug consumers.

Cannabis sativa L. (hemp) native to Central Asia has spread all over the world and is probably the most widely used recreational and illegal drug in the world. Cannabis is a dioecious plant. K-N-B (probably ka-na-ba or qu-nu-bu), the early Sumerian/Babylonian word for cannabis hemp, enters the Indo-Semitic-European language family base, making it one of humankind's longest surviving root words. Already nine or ten thousand years ago the earliest known fabric was woven from hemp. 4700 years ago the first written record of cannabis use is made in the pharmacopoeia of Shen Nung, one of the fathers of Chinese medicine (Li 1974; Abel 1980).

Today about 30,000 publications appeared on the subject of *Cannabis*. In *Cannabis* and its phytochemical products, hashish and marihuana, almost 900 natural compounds to date have been identified. One hundred and twenty of them are so-called cannabinoid compounds (cannabinoids), which are characteristic of and present only in *Cannabis* (Turner et al. 1980; ElSohly & Slade 2005).

To evaluate the type of *Cannabis* plant (e.g. drug type versus fiber type), several classifications based on phenotype were suggested in the past. The phenotype ratio (percentage of cannabinol (CBN)+ percentage of

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*Corresponding author. Email: lumirh@ekmd.huji.ac.il

Δ^9-tetrahydrocannabinol (THC) divided by percentage of cannabidiol (CBD)) was used to differentiate between drug-type and fiber-type cannabis plants. A phenotype ratio greater than 1.0 was classified as drug-type. Less than 1.0 was classified as fiber-type (Grlic 1968; Fetterman et al. 1971).

In the past we studied the influence of climatic, meteorological, agricultural, and ecologic conditions on different types of *Cannabis* cultivated in the same region. We proved that different meteorological conditions during different seasons and in the course of one vegetation period can influence the amount of cannabinoid compounds in the plants, influencing the phenotype of the plants. A *Cannabis* plant cultivated for fibers can become a drug-type plant in a favorable year (Krejčí et al. 1975; Hanuš & Krejčí 1986; Hanuš et al. 1987; Hanuš 1994).

Today it is accepted that if the Δ^9-THC content in the dry flowering tops exceeds 0.3%, *Cannabis* is classified as drug-type (Small & Beckstead 1973; Giroud & Rivier 1996).

In this study, the main cannabinoids (CBD, Δ^9-THC, CBN) in hashish from different police seizures of known origins (Lebanon, Morocco and India) were analyzed at the Department of Natural Products at Hebrew University.

Indian hashish, seized by Czech Republic authorities, was analyzed qualitatively for many cannabinoids and terpenes.

Materials and methods

Origin of hashish

Hashish from different police seizures originated from the three main sources: Lebanon, Morocco, and India.

We tried to evaluate hashish samples according to their geographical origin. Samples were analyzed quantitatively for the three main cannabinoid compounds, CBD, THC, and CBN, and classified according to the phenotype ratio of the appropriate samples.

Fifteen samples of Lebanese hashish (sole – length 15.5 cm, width 8.5 cm), 30 samples of Moroccan hashish (chocolate – lengths 16 cm), and 13 samples of Indian hashish (disk – diameter 2.5 cm), seized by Israeli authorities, and one sample of Indian hashish, seized by Czech Republic authorities, were analyzed quantitatively.

Procedure

For a representative sampling, hashish samples were cut in half and the middle part of these samples was used for analyses. Two hundred milligrams of ground hashish sample was extracted with methanol and filtered through a cotton capillary. The final concentration of extracts was 2 mg/ml of hashish in methanol with 50 μg of an internal standard (tetracosane).

One microliter of this sample was injected to gas chromatography (GC)/mass spectrometry (MS) for analysis.

Instrumentation and conditions of the analysis

For quantitative analysis the samples were analyzed by GC/MS in a Hewlett Packard G 1800B GCD system with a HP-5971 gas chromatograph with an electron ionization detector. The software used was GCD PLUS CHEMSTATION. Column: SPB-5 (30 m × 0.25 mm × 0.25 μm film thickness). Experimental conditions: inlet, 250°C; detector, 280°C; splitless injection/purge time, 1.0 min; initial temperature, 100°C; initial time, 2.0 min; rate, 10°C/min; final temperature, 280°C. Helium flow rate, 1 ml/min.

The qualitative analysis of Indian hashish seized in Czech Republic was carried out using GC/MS of the methanolic extract of the material without derivatization on an Agilent GC 6890 SII_MSD 5973N with ALS. Column DB-5ms (30 m × 0.25 mm × 0.25 μm film thickness). Experimental conditions: inlet, 250°C; detector, 280°C; splitless injection/purge time, 1.0 min; initial temperature, 35°C; initial time, 4.00 min; rate, 7°C/min up to 130°C, then 25°C/min; final temperature, 280°C. Helium flow rate, 1.1 ml/min.

Standards and solutions

Five concentrations from 25.0 to 100.0 μg/ml of CBD, THC, or CBN in methanol were used for calibration curves together with 50.0 μg/ml tetracosane as an internal standard (all concentrations were duplicated). For the other minor cannabinoids, standards and retention times from the Wiley275 library and from our own standards built library, as well as published mass spectra were used. Under the above conditions, CBD and CBC were distinctively separated.

Results

Hashish worked-up in different external appearances – big "sole", thin "chocolate", and "disk" – were analyzed quantitatively for the content of three main cannabinoid compounds (CBD, Δ^9-THC, and CBN) with the help of GC/MS. After separation, it was possible to identify other minor cannabinoids. As it was not the aim of this work, only several other cannabinoids, easily visible on chromatograms, were identified qualitatively (CBDV, Δ^9-THCV, CBE, Δ^8-THC, CBGM, and CBG).

The amounts of the main cannabinoids in hashish are presented (see Table 1).

The appropriate cannabinoid acids, which are thermally unstable, are original cannabinoids found in the plant from which the neutral cannabinoids originate by decarboxylation during ripening, drying and storage of

Table 1. Cannabinoid content in seized hashish.

CBD (%)	Δ^9-THC (%)	CBN (%)	Δ^9-THC + CBN /CBD	Country of origin
12.79	4.20	2.08	0.49	Lebanon
11.01	3.85	1.78	0.51	Lebanon
6.48	3.68	0.72	0.68	Lebanon
11.47	3.37	1.71	0.44	Lebanon
10.84	2.92	1.94	0.45	Lebanon
7.70	2.59	1.43	0.52	Lebanon
10.72	2.44	2.00	0.41	Lebanon
11.42	2.32	1.79	0.36	Lebanon
8.17	2.29	1.17	0.42	Lebanon
7.76	1.76	1.05	0.36	Lebanon
6.38	1.62	0.96	0.40	Lebanon
8.55	1.36	1.69	0.36	Lebanon
6.33	1.29	1.79	0.49	Lebanon
5.69	1.07	1.16	0.39	Lebanon
9.42	0.93	2.20	0.33	Lebanon
5.08	13.41	1.36	2.91	Morocco
4.76	12.57	2.88	3.25	Morocco
4.70	12.38	1.57	2.97	Morocco
4.17	12.14	1.94	3.38	Morocco
3.95	11.49	1.67	3.34	Morocco
4.04	10.96	2.61	3.36	Morocco
3.69	10.79	2.73	3.67	Morocco
3.24	10.58	2.31	3.98	Morocco
4.99	10.42	2.72	2.63	Morocco
4.38	10.37	1.19	2.64	Morocco
4.41	10.18	1.12	2.56	Morocco
4.10	9.93	1.59	2.81	Morocco
2.87	9.79	1.05	3.76	Morocco
3.63	9.66	2.27	3.29	Morocco
2.98	9.35	1.23	3.22	Morocco
3.87	9.10	1.29	2.69	Morocco
4.81	9.07	2.53	2.41	Morocco
3.64	8.85	1.34	2.80	Morocco
4.49	8.82	0.91	2.17	Morocco
2.89	8.63	1.42	3.48	Morocco
2.67	8.52	1.40	3.71	Morocco
5.14	8.01	2.67	2.08	Morocco
4.45	7.99	1.89	2.22	Morocco
4.25	7.73	2.47	2.40	Morocco
4.32	7.61	2.41	2.32	Morocco
3.32	6.46	2.94	2.83	Morocco
1.73	5.82	0.71	3.77	Morocco
1.52	5.40	0.65	3.98	Morocco
1.70	5.35	0.98	3.73	Morocco
1.76	5.08	0.93	3.41	Morocco
7.45	16.45	3.33	2.66	India
10.29	14.74	2.15	1.64	India
3.85	7.50	2.32	2.55	India
7.72	6.05	4.80	1.41	India
3.44	5.94	5.82	3.42	India
2.15	4.30	4.91	4.28	India
13.13	4.28	2.74	0.54	India
1.54	4.27	3.38	4.96	India
3.33	3.66	5.86	2.86	India
0.78	2.09	2.82	6.30	India
2.31	0.53	2.15	1.16	India
2.61	Traces	1.30	0.50	India
1.08	Traces	1.61	1.49	India

the samples. They are also decarboxylated after injection to the gas chromatograph.

Several typical chromatograms with identified cannabinoids in hashish are shown in Figures 1–5. These samples of known origin from Lebanon, Morocco, and India were compared for cannabidiol (Figure 6), Δ^9-tetrahydrocannabinol (Figure 7) and cannabinol (Figure 8) content

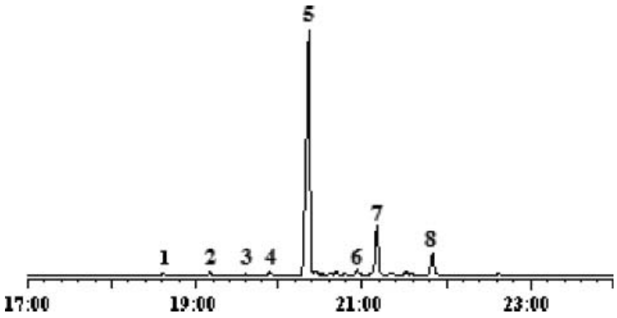

Figure 1. Hashish sample (sole) from Lebanon (RL107).
Key: 1, cannabidivarin (CBDV); 2, cannabicitran; 3, Δ^9-tetrahydrocannabivarol (Δ^9-THCV); 4, mw 314; 5, cannabidiol (CBD; 17.24%); 6, cannabielsoin (CBE); 7, Δ^9-tetrahydrocannabinol (Δ^9-THC; 5.53%); 8, cannabinol (CBN; 2.78%).

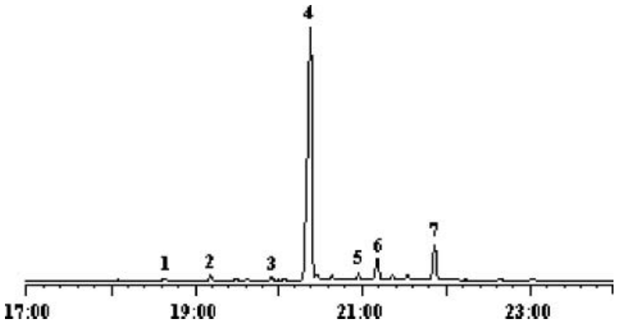

Figure 2. Hashish sample (sole) from Lebanon (RL110).
Key: 1, cannabidivarin (CBDV); 2, cannabicitran; 3, mw 314; 4, cannabidiol (CBD; 9.42%); 5, cannabielsoin (CBE); 6, Δ^9-tetrahydrocannabinol (Δ^9-THC; 0.93%); 7, cannabinol (CBN; 2.20%).

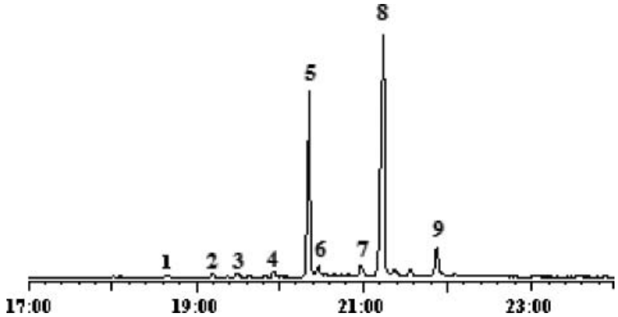

Figure 3. Hashish sample (chocolate) from Morocco (RL128). Key: 1, cannabidivarin (CBDV); 2, cannabicitran; 3, Δ^9-tetrahydrocannabivarol (Δ^9-THCV); 4, mw 314; 5, cannabidiol (CBD; 2.98%); 6, cannabichromene (CBC); 7, cannabigerol monomethyl ether (CBGM); 8, Δ^9-tetrahydrocannabinol (Δ^9-THC; 9.35%); 9, cannabinol (CBN; 1.23%).

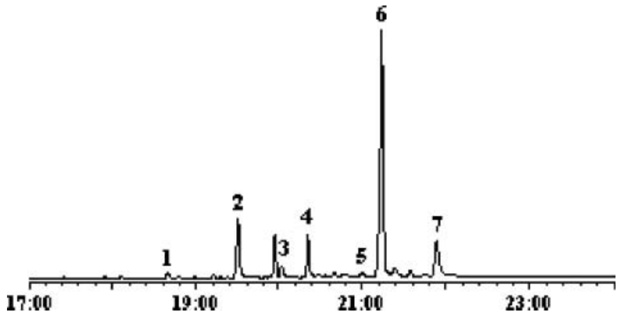

Figure 5. Hashish sample (disk) from India (RL153). Key: 1, cannabidivarin (CBDV); 2, Δ^9-tetrahydrocannabivarol (Δ^9-THCV); 3, cannabivarin (CBV); 4, cannabidiol (CBD; 0.74%); 5, Δ^8-tetrahydrocannabinol (Δ^8-THC); 6, Δ^9-tetrahydrocannabinol (Δ^9-THC; 11.70%); 7, cannabinol (CBN; 1.98%).

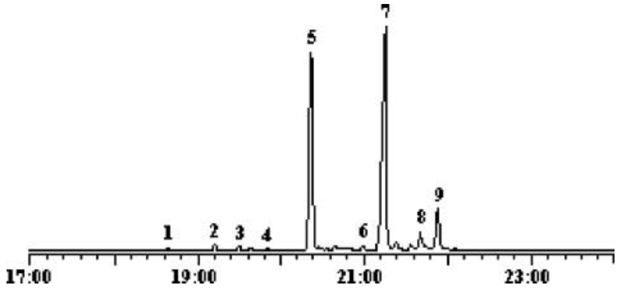

Figure 4. Hashish sample (disk) from India (RL142). Key: 1, cannabidivarin (CBDV); 2, cannabicitran; 3, Δ^9-tetrahydrocannabivarol (Δ^9-THCV); 4, mw 314; 5, cannabidiol (CBD; 7.45%); 6, Δ^8-tetrahydrocannabinol (Δ^8-THC); 7, Δ^9-tetrahydrocannabinol (Δ^9-THC; 16.45%); 8, cannabigerol (CBG); 9, cannabinol (CBN; 3.33%).

and ranked in descending order based on the amounts of these three cannabinoids.

Finally, each sample was evaluated according to the ratio of the three main cannabinoids (THC + CBN/CBD) and samples of different known origin were compared (Figure 9).

In Figure 10, samples of Moroccan and Lebanese origin are clearly distinguished.

Several papers exist showing that more chemicals must be considered – e.g. the major and minor cannabinoids, the terpenic compounds – in order to characterize the cannabis plant.

Hashish coming from India seized in the Czech Republic in July 2015 was analyzed for terpenes as well as for cannabinoids. This hashish was very dark, very soft and sticky. Quantitative analysis revealed 20.85% Δ^9-THC, 0.23% CBD, and 0.12% CBN. The next cannabinoids in higher concentration were Δ^9-THCV, CBG, and CBDV. Altogether 42 terpenes (another 14 terpenes were not identified with certainty) and 20 cannabinoids (three of them new; another 13 cannabinoids were not identified with certainty) were identified in this hashish. The main monoterpenes were myrcene, α-pinene, and limonene; the main sesquiterpenes were β-caryophyllene, α-humulene, and valencene. All identified terpenes were α-thujene, α-pinene, camphene, thuja-2,4(10)-diene, β-pinene, myrcene, α-phellandrene, Δ^3-carene, α-terpinene, limonene, *cis*-β-ocimene, *trans*-β-ocimene, γ-terpinene, terpinolene, *p*-cymenene, linalool, 1,3,8-*para*-menthatriene, *exo*-fenchol, *trans*-pinene hydrate, *trans*-pinocarveol, borneol, 1,8-menthadien-4-ol, α-terpineol, *trans*-carveol, citronellol, *trans*-ocimenone, geraniol, *trans*-sabinyl acetate, α-cubebene, piperitenone oxide, α-ylangene, α-copaene,

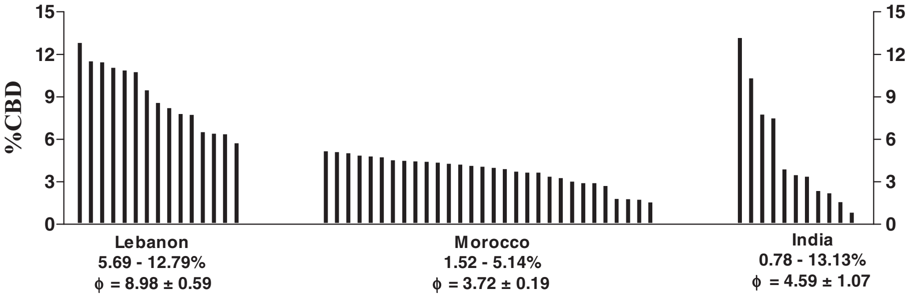

Figure 6. Cannabidiol content in the samples of known origin (samples arranged according to the descending content of CBD).

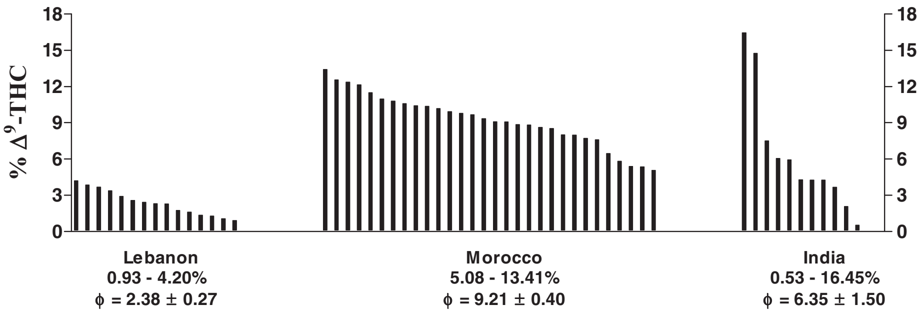

Figure 7. Δ^9-Tetrahydrocannabinol content in the samples of known origin (samples arranged according to the descending content of Δ^9-THC).

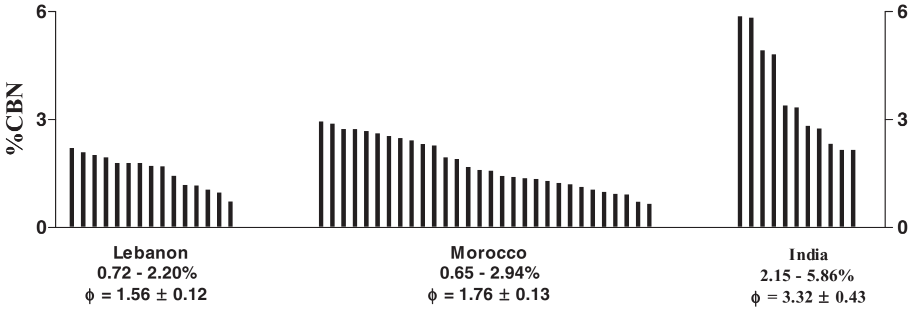

Figure 8. Cannabinol content in the samples of known origin (samples arranged according to descending content of CBN).

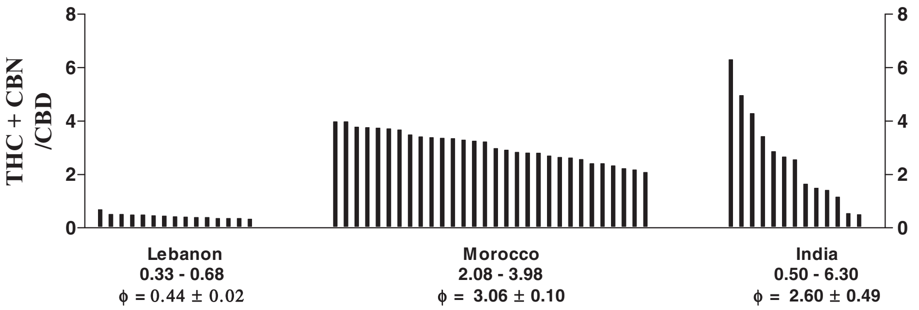

Figure 9. Phenotype of the samples of known origin (samples arranged according to descending value of the phenotype) with three different evaluations used.

β-bourbonene, *cis*-caryophyllene, β-caryophyllene, *trans*-β-farnesene, α-humulene, γ-muurolene or α-amorphene, valencene, *cis*-α-bisabolene, *cis*-nerolidol, selina-3,7(11)-diene, caryophyllene oxide, caryophylla-4(12),8(13)-dien-5-ol, and α-bisabolol. The identified cannabinoids included (in parentheses % relative to the main cannabinoid, Δ^9-THC) cannabidiorcol (CBD-C1), 0.015%; cannabiorcochromene (CBC-C1) (new cannabinoid, Figure 11), 0.011%; GC-MS *m/z* 258 (M^+, 7.7 %), 243 (2.5%), 207 (2.8%), 176 (13.5%), 175 (100%), 174 (5.9%), 103 (3.9%), 69 (3.8%), 44 (3.3%), 41 (7.4%); Δ^9-tetrahydrocannabiorcol (Δ^9-THC-C1), 0.390%; cannabidivarol (CBDV), 5.053%; cannabivarichromene (CBC-C3), 0.544%; *cis*-Δ^9-tetrahydrocannabivarol (*cis*-Δ^9-THCV) (new cannabinoid, Figure 12), 0.145%; GC-MS *m/z* 286 (M^+, 65.8%), 271 (78.1%), 244 (12.4%), 243 (63.3%), 204 (16.9%), 203 (100%), 201 (11.4%), 174 (12.2%), 165 (19.3%), 91 (16.7%), 43 (13.7%), 41 (19.7%); cannabicitran (CBCT), 0.202%; Δ^9-tetrahydrocannabivarol (Δ^9-THCV), 36.326%; Δ^9-tetrahydrocannabinol-C4 (Δ^9-THC-C4), 0.062%; cannabivarol (CBN-C3), 2.438%; cannabidiol (CBD), 31.448%; cannabichromene (CBC), 0.214 %; CBD or THC mono-*n*-propyl ether, 1.503%; *cis*-Δ^9-tetrahydrocannabinol (*cis*-Δ^9-THC), 0.974%; cannabielsoin (CBE), 0.315;, Δ^9-tetrahydrocannabinol (Δ^9-THC), 100.000%; cannabinerol (new cannabinoid, Figure 13), 0.296%; GC/MS *m/z* 316 (32.85%), 259 (12.64%), 233 (16.70%), 231 (56.66%),

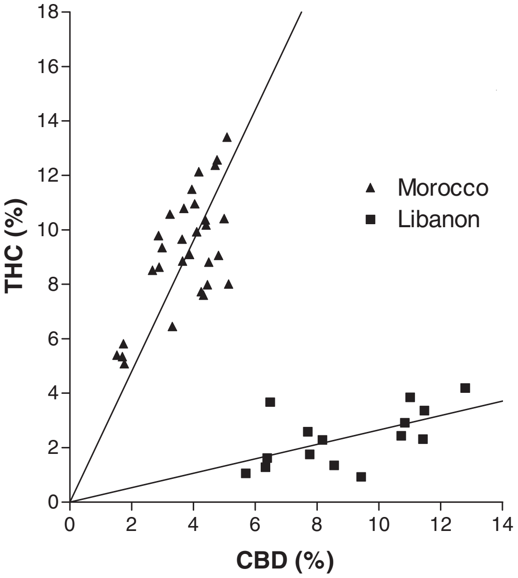

Figure 10. Plot of Δ^9-tetrahydrocannabinol (THC) % vs. cannabidiol (CBD) % for hashish samples of Moroccan and Lebanese origin. Linear regression lines (forced through the origin) are drawn for each origin.

194 (15.39%), 193 (100.00%), 136 (17.58%), 123 (20.39%), 121 (12.04%), 43 (21.07%), 41 (27.34%); cannabigerol (CBG), 17.415%; cannabinol (CBN), 6.188%; and cannabifuran (CBF), 0.359%.

Discussion

Unexpectedly, it was not easy to compare our result with the published ones.

Different batches of cannabis resin from Lebanon were differentiated by comparing the principal cannabinoid contents – CBD, CBDA, CBN, and THCA (McDonald & Gough 1984). Jenkins and Patterson (1973) studied relations between chemical composition and geographical origin of cannabis. In cannabis of Moroccan origin, CBD, THC, and CBN were quantified. Cannabis plants from seeds of Moroccan origin were cultivated in the UK. In resin from Morocco illicitly imported to the UK the THC was quantified (Pitts et al. 1990). Quantitative determination of the average levels of Δ^9-THC content in cannabis (leaves and inflorescences) in 180 fresh male and female plants (0.1−2.2%), 52 dry female plants (0.2−7.5%) and 13 powdered plants (5.5−11.3%) was published by Stambouli et al. (2005). Male and female plant material (e.g. marihuana) was evaluated for CBD and THC content (Taylor 1985). The aim to estimate the geographical origin of cannabis samples based on hydrocarbon content (Mobarak et al. 1974), cannabinoids content (Krejčí et al. 1975; Idilbi 1985a, 1985b) and complex chemical profiles (cannabinoids and non-cannabinoids) (Brenneisen & ElSohly 1988) can be found in the literature.

The stability of *Cannabis sativa* L. samples (charas, ganja, and bhang) and their extracts on prolonged storage

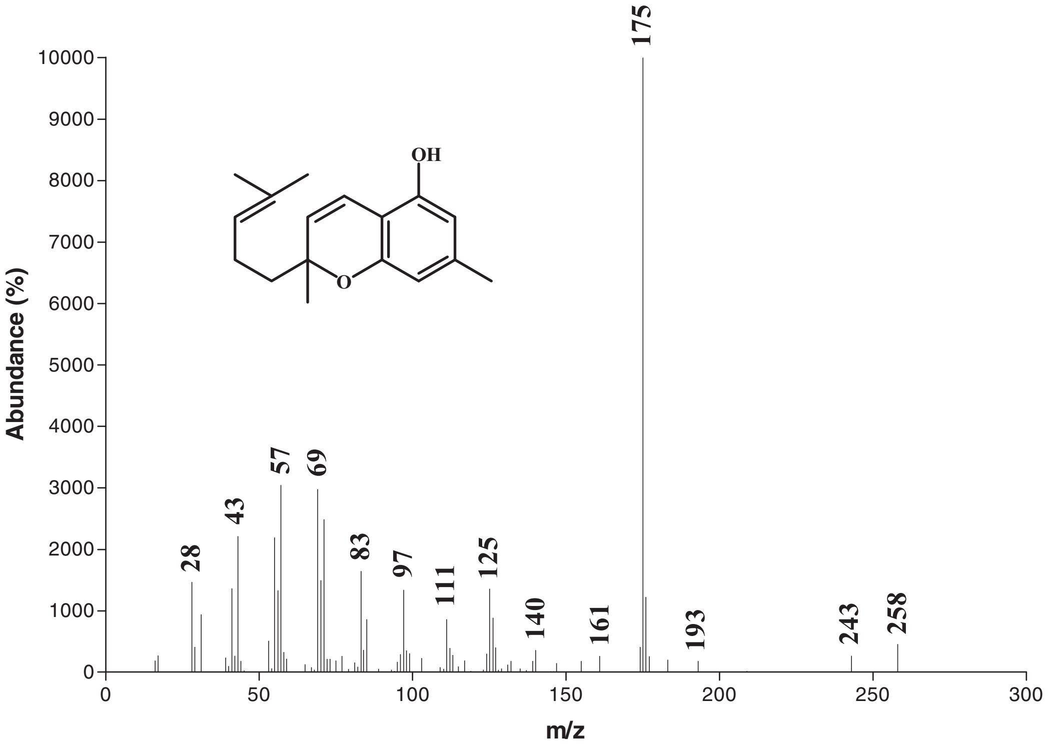

Figure 11. Mass spectrum of cannabiorcochromene (CBC-C1).

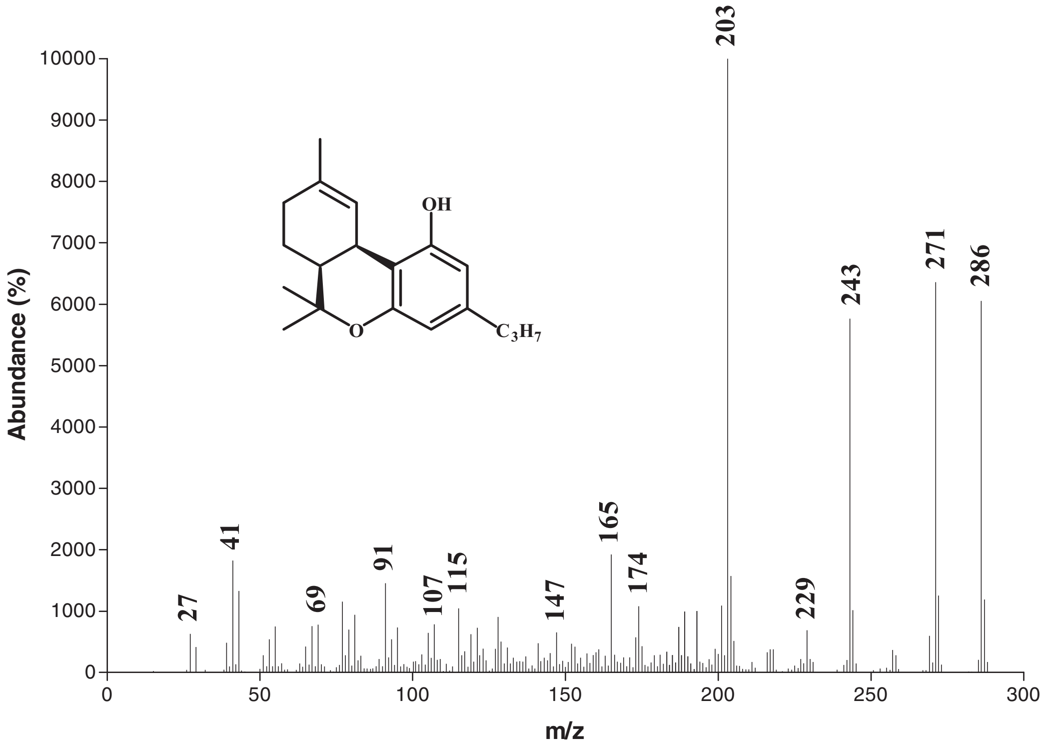

Figure 12. Mass spectrum of *cis*-Δ^9-tetrahydrocannabivarol (*cis*-Δ^9-THCV).

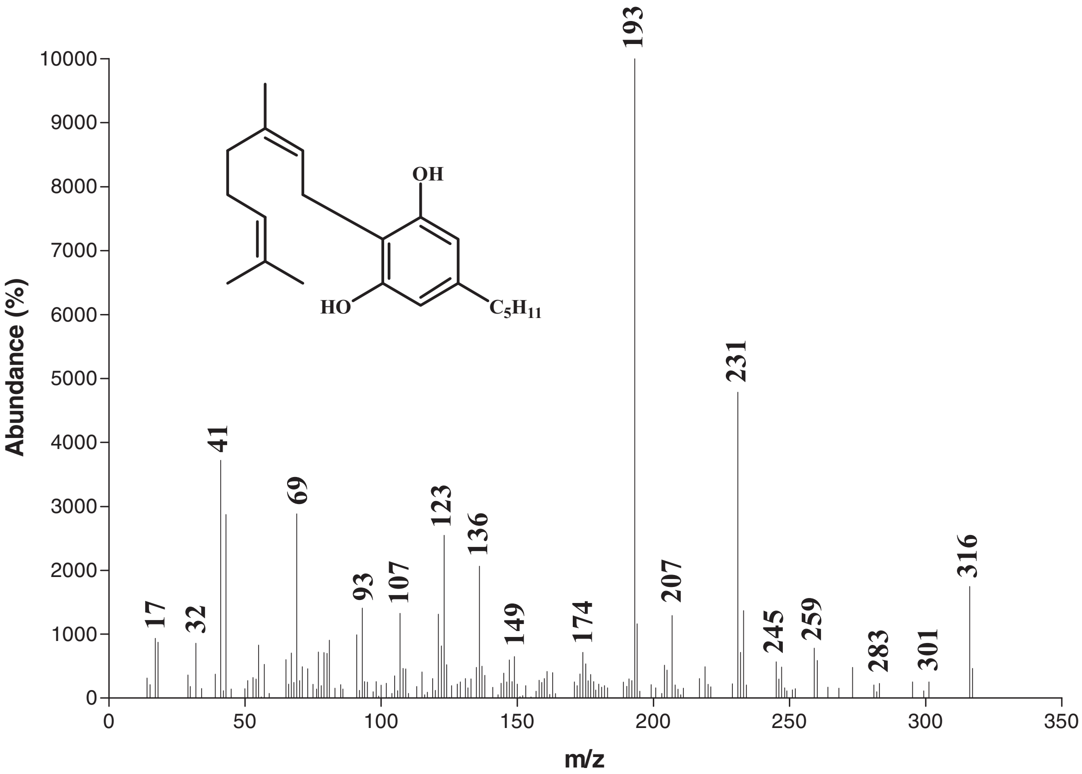

Figure 13. Mass spectrum of cannabinerol.

was studied in India (Narayanaswami et al. 1978). In all samples CBD, THC and CBN were quantified. Wild cannabis from different altitudes and locations collected in northern India was analyzed quantitatively for 10 different cannabinoids. The data obtained were compared with data obtained from the same variants grown in Mississippi (Turner et al. 1979).

Ohlsson et al. (1971) quantified cannabinoid constituents (CBD and THC) of male and female *Cannabis sativa* from Lebanon and Morocco.

Variation in the THC content in illicitly imported *Cannabis* products of Lebanon, Morocco and India origin was determined (Baker et al. 1980, 1982). The same authors compared samples' chemical features (CBD and THC content) in cannabis plants grown in the UK from seeds of Moroccon and India origin (Baker et al. 1982, 1983).

Most of the hashish samples were of Lebanese, Moroccan, and Indian origin. Analysis of these samples gave us the ability to compare the main cannabinoid amounts in samples of these three countries (Table 1). The chemotype appears to be genetically determined, while the individual cannabinoid concentrations can be influenced by ecological factors.

Cannabidiol content (Figure 6) is high in hashish of Lebanese origin and low in Moroccan ones. Samples from India showed a spectrum from high to low CBD content. In all hashish samples of known origin CBD varied from 0.78% to 13.13%. Δ^9-Tetrahydrocannabinol content (Figure 7) is low in hashish of Lebanese origin and high in Moroccan ones. Samples from India showed again a spectrum from high to low Δ^9-THC content. The variation of Δ^9-THC content was from traces to 16.45%. Cannabinol content (Figure 8) is almost the same in all of the samples of Lebanese and Moroccan origin and higher in hashish of Indian origin. All samples varied from 0.65% to 5.86% of CBN. Various amounts of cannabinol in the samples are caused by the aging of the sample, the storage conditions, and the type of hashish (Ross & ElSohly 1997–1998; Martone & Della Casa 1990). Cannabinol is obviously a degradation product of THC (Hanuš et al. 1985).

Phenotypic index is compared in Figure 9. Hashish of Lebanese origin is indicative of fiber-type phenotype and Moroccan the drug-type phenotype. The only information we found in the literature (Idilbi 1985b) concerning the phenotypic index of hashish of Lebanese origin – 0.465 – is in agreement with our results (0.33–0.68). In hashish of Lebanese origin, we also detected cannabielsoin. Hashish of Indian origin shows different hashish types from the fiber- to drug-type phenotypes. Phenotypic index and the amounts of the three main cannabinoids in the sample is very useful information regarding the country of origin, but one must take into account the history of the sample (country of origin, type of the plant [cultivated for fibers or for drug abuse], origin of the cultivated seeds, climatic and ecologic conditions the year of cultivation and the age of the analyzed sample), all of which are usually unknown in seized samples.

Some samples of Indian origin showed higher amounts of propylcannabinoids such as Δ9-tetrahydrocannabivarol (THCV) or cannabivarin (CBV). Cannabielsoin was identified in the samples of Lebanese origin. To identify the country of origin of hashish samples, the three main cannabinoids have only informative value and it appears other compounds should be taken into account for this purpose (for example terpenoid variation as we did for the sample seized in the Czech Republic). Nevertheless, even in this small group of samples, significant differences were observed.

As there is no generally used method for the comparison of samples (exact conditions for how to work up the samples for analysis), we were unable to compare our results with those published. Nevertheless, our results showed valuable data for the comparison of resins of different sample origins.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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