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THE ESSENTIAL OIL OF *CANNABIS SATIVA*

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Abstract

In previous reports the presence of cannabinoids in the distilled essential oil of Cannabis sativa *L. was proved, besides the presence of mono- and sesquiterpene hydrocarbons.*

In this paper the localization of the cannabinoids in the glandular hairs of the leaves and with that the possible biogenetic relation with the components of the essential oil are demonstrated by microscopic examination after colouring tests and gaschromatographic analysis of the isolated contents of individual glandular hairs. Quantitative data about the relation between essential oil and cannabinoids are obtained by comparing the extracts without and after preceding steam distillation.

On acount of the origin of the seed (birdseed), special attention was paid to the botanical description of the plant material and to the counting of chromosomes.

Introduction

In the essential oil of *Cannabis sativa* L., obtained by steam distillation of the dried herb, small amounts of cannabinoids turn out to be present. By means of a combination of TLC and GLC MALINGRÉ et al. (1973) demonstrated the presence of at least the following cannabinoids: cannabidivarol (CBD-C3), tetrahydrocannabivarol (Δ′-THC-C3), cannabivarol (CBN-C3), cannabidiol (CBD-C5), Δ′-tetrahydrocannabinol (Δ′-THC-C5) and cannabinol (CBN-C5).

Besides these cannabinoids a considerable number of mono- and sesquiterpene hydrocarbons were proved by HENDRIKS et al. (1975). It is interesting to know from a biogenetic point of view if cannabinoids in the plant are localized entirely or mainly in the essential oil containing glandular hairs. To get a first impression about the localization, leaf material is treated by well-known colouring reagents on cannabinoids and subsequently examined (photo)-microscopically.

Besides, the composition of the contents of the glandular hairs is examined by means of a technique used before at the analysis of the essential oil of *Mentha aquatica* L. (MALINGRÉ et al., 1969). With this technique individual glandular

hairs are pricked by means of a glass capillary, supported by a micromanipulator. The contents of the glass capillary is analysed gaschromatographically in a direct way by special injection techniques.

To gather more quantitative data about the presence of the cannabinoids in the distilled oil in comparison with the quantities in the plant material, one part of a homogeneous sample of cannabis leaves is directly extracted and another part extracted after steam distillation. The composition of both extracts is compared with that of the distilled oil by means of gaschromatography. Because this investigation and previous ones (DE ZEEUW, MALINGRÉ and MERKUS, 1972) were carried out with plants obtained by cultures of the same strain of *Cannabis* (X), of which the ecologic origin is not clear (birdseed), a floristic description of the plant material will be framed and the results of the countings of chromosomes will be given.

Experimental

Plant material

Samples of *Cannabis sativa* „strain X" were collected from flowering plants, grown at the experimental garden at Buitenpost (The Netherlands). Plants were cultivated in the open air for three years from seeds of the same strain, originally obtained as birdseed.

Description: erect, branched, herbaceous, annual, dioecious, fertile plant, reaching up to 3 m in height. Root; stout, spindle-shaped, branched tap root, up to 25 cm in length and 6 cm in diameter. Stem; erect, coarse, green, at the base massive and round in cross section, more upwards hollow and obtusely polygonal by longitudinal ribs, covered with unicellar cystolithic trichomes and few glandular trichomes. Leaves; decussate near the base, alternate, towards the apex and on the branches, (3–)5–7–9–(11–) palmatisect. Petioles; up to 8 cm long with a narrow groove along the upper side. Leaflets; uneven in size with the largest up to 25 cm, narrowly lanceolate, acute, coarsely serrate, soft-textured, venation pinnate, the upper surface dark green, the lower surface contrasting pale green. Covering trichomes; conical and unicellar, cystolithic or not cystolithic, short (–150 μ) and enlarged at the base (upper surface) or more elongated (–250 μ) and less enlarged at the base (lower surface). Glandular trichomes; multicellar, multiseriate stalk and multicellar head (–55 μ in diameter) or uniseriate stalk of one or two cells and 1-4-cellar head (–25 μ in diameter). Female inflorescense; racemose, compact, short and few-flowered, not projecting beyond the leaves. Individual pistillate flower with green tubular bract about 3.5–4 mm long, out of which project two long slender stigmas with a length of 7–8 mm. The bract is covered with hairs and short-stalked or stalkless glands. Male inflorescense; paniclous, loosely arranged, greatly branched, many flowered, standing out from the leaves. Individual staminate flower with 5 greenish-white minutely hairy sepals about 4 mm long and 5 pendulous stamens with a length of 4 mm. Pollen grains; oblate, 35 μ in diameter, with 2–4 germ spores. Fruit; achene, ellipsoid, greyish-brown, reticulately veined, rimmed by a keel, 4.3–4.6 mm long, 2.8–3.4 mm in diameter.

Counting of chromosomes

Chromosome counts were made from preparations of roottips of germinating seeds, using the squash technique. The preparations were prefixed in a mixture of 1% of chromic acid: $HOAc$:

formalin (10 : 1 : 4) for a period of 2 or 3 hrs and subsequently coloured for 15 minutes in a lacto-orcine solution (1 g orcine, 9.4 ml lactic acid, 24.4 ml $HOAc$, 16.2 ml H_2O; reflux for 1 hr and filter) and finally squashed in 45% $HOAc$. Chromosomes were counted with the aid of a drawing prism.

Colouring tests

Parts of the fullgrown leaves of male and female plants were cleared, subjected to different colouring agents and subsequently examined photomicroscopically.

Beamtest (KORTE and SIEPER, 1964); In order to combine clearing and colouring a 30% aqueous solution of KOH was used instead of a 5% ethanolic solution. After heating for 1 minute a blue colour was observed.

Fastblue test (SEGELMAN, 1973); To combine the clearing and colouring process the plant material was treated with a newly prepared solution of 0.1%. Fast blue salt B in chloral hydrate solution. Red-purple colours appeared after heating for 1 minute.

Isolation of the contents from individual glandular hairs

By means of a stereomicroscope and a micromanipulator, equipped with a glass capillary, about ten glandular hairs of one leaf were pricked. The contents of the glass capillary were directly injected by special techniques into the injection port of a gaschromatographic apparatus. The contents of random leaf cells, including covering trichomes, are isolated and analysed in this way as well.

Isolation of the essential oils by steam distillation

360 g of fresh leaves were homogenized and dried at the air to a weight of 120 g. Of this sample 9/10 part (108 g) was subjected to steam distillation for 4 hours according to the method as described in the Dutch Pharmacopoeia (1966), resulting in 0.13 ml of essential oil.

Isolation of the cannabinoids by extraction

12 g of the same sample of dried leaves were extracted by shaking with five portions of 60 ml of light petroleum (b.p. 40°), followed by filtration to remove plant materials. The combined extracts were evaporated to dryness in a flash evaporator and the oily residue was redissolved in 1 ml of petroleum ether. In the same way 1/9 part ($\sim$ 12 g of dried leaves) of the dried residue of the plant material after steam distillation was extracted.

Gas-liquid chromatography

A Hewlett Packard 5750 instrument with dual flame ionization was used. Glass columns, 3 mm$\times$2 m, packed with 3% OV 17 on chromosorb G-HP, 100–120 mesh. Carrier gas nitrogen 50 ml/min. Injection block 290°, oven 100–280°, 6°/min, detector 290°. Sample size 3 µl, attenuator 8×10^4 (distilled oil) and 4×10^3 (extracts). Isolated contents of $\pm$10 glandular hairs; oven 225°, attenuator 2$\times$1.

Results and Discussion

The culture of *Cannabis sativa,* strain no X, grown for a period of three years, but every year from seeds of the previous year, shows no botanical variation. The

botanical description of the plant material, as given in the experimental section, is for the greater part in agreement with the descriptions as given, among others, by STEARN (1970) and MILLER (1970). Also the result of the counting of the chromosome numbers (2n=20) corresponds with literature data (Chromosome-Atlas, 1955 and MILLER, 1970). By colouring with the modified Beam test and the Fastblue salt test and subsequent photomicroscopic examination, it is demonstrated that the cannabinoids are mainly localized in the glandular hairs (Fig. 1). Not only the glandular hairs but also the centre of the leaf veines are slightly coloured by the Fast blue salt reagent. It is remarkable that pollengrains are coloured in the same way as the glands. Although the plant material as such was positive with the Meta-Duquenois test (DE FAUBERT-MAUNDER, 1969) it was impossible to colour glands or other parts of the leaf with this reagent (vanillin + acetaldehyde), in spite of different variations. If in this way it is demonstrated that cannabinoids are mainly localized in the glandular hairs and furthermore the presence of small amounts of cannabinoids in the distilled oil is proved, it is important to analyse the contents of the glandular hairs.

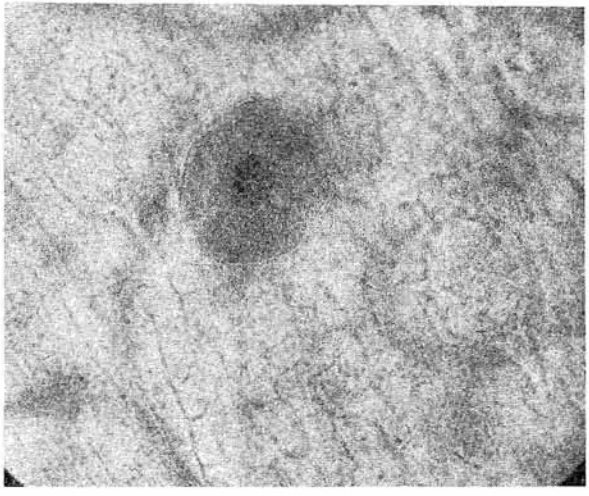

Fig. 1. *Cannabis sativa:* colouring of glandular trichomes with Fast blue salt B reagent (400×)

Quantitative evaluation of the gaschromatogram of the contents of about ten glandular hairs isolated by pricking shows hat more than 90% of the contents consists of cannabinoids. Furthermore it appears by means of this method that no cannabinoids are detectable in the contents of other random leaf cells and covering trichomes.

Quantitative approximation of the chromatograms of the extracts (Table I) learns that the chromatogram of the extract without preceding steam distillation contains about 80% cannabinoids and the chromatogram of the extract after pre-

ceding distillation about 90% cannabinoids. Comparison of the composition of both extracts shows that about ¼ of the „essential oil" part is not removed by steam distillation and learns that the total amount of cannabinoids remains about constant or only sligthly diminishes. This is in agreement with the calculation of the percentage of the primary amount of cannabinoids that moved to the essential oil during distillation, that is less than 1%.

Table I

Gaschromatographic analysis of the extracts and the essential oil of *Cannabis sativa*

Peaknumber	Extract^{+}	Extract^{-}	Essential oil
	%	%	%
1	6.4	0.8	22.2
2	3.8	0.5	18.7
3	2.8	0.5	16.6
4	4.3	0.6	16.3
5	0.6	–	5.8
6	0.4	–	8.3
7	1.7	0.5	8.8
8	–	1.8	–
9	–	0.7	–
10	0.5	0.3	0.4
11	1.3	2.9	0.4
12	0.3	0.3	–
13	0.3	0.7	–
14	4.1	6.3	0.4
15	3.2	2.3	–
16	65.0	74.1	2.0
17	4.8	6.8	0.2
18	0.6	0.9	–

Peaknumbers 1–11 represent the terpenoid components.
Peaknumbers 12–18 represent the cannabinoid components.
$^{+}$; extraction without preceding steam distillation.
$^{-}$; extraction after preceding steam distillation.
11 = caryophyllene oxide, 14 = CBD, 16 = THC, 17 = CBN.

The great difference between the percentage of cannabinoids in the essential oil obtained respectively by steam distillation and pricking glands is due to the low volatility of the cannabinoids.

These results support the conclusion that cannabinoids are mainly localized in the glandular hairs. The observations of De Pasquale (1974) under the electron microscope have made it evident that the resin-producing cellular elements are to be found almost exclusively in the head of the hairs and in the apex part of the stalk.

The terpene hydrocarbons of the essential oil and the cannabinoids with a partly terpene structure both present in the glands of *Cannabis sativa* at the same time suggest a biogenetic relation between these components. The presence of cannabinoids in very young leaves, as proved by RASMUSSEN and HERWEYER (1975) suggests a relation in an early state of plant development.

To confirm this possible relation further biogenetic investigation will be made.

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