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QUANTITATIVE ANALYSIS OF CANNABINOIDS IN THE SECRETORY PRODUCT FROM CAPITATE-STALKED GLANDS OF CANNABIS SATIVA L. (CANNABACEAE)

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Capitate-stalked glandular trichomes from pistillate bracts of *Cannabis sativa* L. were analyzed to determine the cannabinoid composition of secretory products within the secretory sac. Analyses were performed on cloned materials of a strain characteristically high in cannabidiol. The secretory product is accumulated in a single large secretory sac, which develops above a multicellular disk of secretory cells during gland ontogeny. The content of the secretory sac was removed with micropipets, using a micromanipulator without damaging or removing disk cells or their contents, and analyzed by gas-liquid chromatography. The cannabinoid content of the secretory sac was compared with previously quantitated cannabinoids of whole capitate-stalked glands. Results indicated that nearly all of the cannabinoid content of capitate-stalked glands was present in the secretory sac.

Introduction

Cannabis sativa L. contains a group of terpenophenolic compounds, termed cannabinoids, which include the major psychoactive compound, Δ^9-tetrahydrocannabinol (Δ^9-THC), and the several related compounds cannabidiol (CBD), cannabinol (CBN), and cannabichromene (CBC) (MECHOULAM 1973). Various strains of *Cannabis* possess different ratios of these cannabinoids (SMALL and BECKSTEAD 1973). Several studies have implicated the glandular trichomes as the site of cannabinoid accumulation (FUJITA et al. 1967; DEPASQUALE 1974; ANDRÉ, LAUWEREYS, and VERCRUYSEE 1975; MALINGRÉ et al. 1975; TURNER, HEMPHILL, and MAHLBERG 1977, 1978).

Three types of glandular trichomes occur on pistillate plants: bulbous, capitate-sessile, and capitate-stalked forms (HAMMOND and MAHLBERG 1973). Bulbous and capitate-sessile types occur on both vegetative and floral shoots; capitate-stalked glands occur only on bracts and related floral leaves of pistillate plants (HAMMOND and MAHLBERG 1977).

Capitate-sessile and capitate-stalked glands produce secretory products which accumulate in a single large distended secretory sac bounded by a sheath. Development of the secretory sac occurs through separation of wall layers within the outer wall of the gland disk cells (HAMMOND and MAHLBERG 1978). Although the ultrastructure of these glands has been studied (HAMMOND and MAHLBERG 1978), it is incompletely understood how secretion takes place or where the secretory products occur in the plant. Studies on *Cannabis* in our laboratory (TURNER et al. 1977, 1978, 1980) continue in an effort to understand the process of cannabinoid production, specifically in relation to glandular trichomes.

Several investigations on cannabinoids in the glandular trichomes of *Cannabis* have utilized micromanipulative procedures to collect samples for analysis. MALINGRÉ et al. (1975) reported that the contents of glands from the leaf consisted of 90% cannabinoids, although they did not indicate whether their samples represented sac contents or whole glands. ANDRÉ et al. (1975) qualitatively reported cannabinoids in glands, but also did not specify the origin of their samples to be from whole glands or the secretory sac. FUJITA et al. (1967) analyzed samples referred to as the "oily part" and the "head" of the gland, but detected cannabinoids only in the "head" sample.

The purpose of this study is to quantitate the cannabinoids present in the secretory sac of capitate-stalked glands. Micromanipulative techniques are combined with gas-liquid chromatographic analyses to examine the secretory sac of the gland as a major site for cannabinoid localization.

Material and methods

SOURCE OF GLANDS.—Floral bracts were collected from a mature pistillate clone of *Cannabis sativa* L. (TURNER et al. 1977). Collections were made at various times from August to February from clone 87, a fiber strain characteristically high in cannabidiol. Bracts selected for sampling of glandular trichomes were 4–9 mm long. Capitate-stalked glands, which were refractile in appearance when viewed under the microscope, were routinely selected for sampling of the secretory product.

SAMPLE PREPARATION.—Bracts were impaled on an insect pin and affixed to a microscope slide. The insect pin was equipped with a device enabling rotation of the bract through 360°. The microscope slide was mounted on the stage of an inverted microscope mounted on a Leitz micromanipulator. Siliconized glass micropipets, 15–30 μm in diameter

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at the tip, were used to collect the contents of the secretory sac of individual capitate-stalked glands on bracts. The micropipet tip was then crushed into a vial containing 0.5 ml of "spectranalyzed" grade chloroform.

GAS-LIQUID CHROMATOGRAPHY (GLC).—Samples were evaporated to dryness under nitrogen and redissolved in 100 μl of chloroform containing eicosane as an internal standard. Analyses were performed on a Hewlett-Packard 5710A chromatograph equipped with a hydrogen flame ionization detector and a Hewlett-Packard 3380A integrator. Glass columns (2 mm ID × 2.43 m) were treated with 5% dimethyldichlorosilane in toluene and packed with 3% OV-1 on 80/100 mesh Supelcoport. The inlet and detector temperatures were 250 C and 300 C, respectively. A 1-μl quantity of sample was injected and analyzed with a program of 200–240 C at 2 C/min with an additional 8-min isothermal period at 240 C. Nitrogen was used as the carrier gas with a flow rate of 20 ml/min.

Results and discussion

Twenty-six secretory product samples were collected from gland secretory sacs for analysis during a 7-mo period from August to February. Analysis of each sample by GLC indicated the presence of cannabinoids in the secretory product. The characteristic cannabinoid in this clone, CBD, averaged 59.4 ± 22.8 ng/gland and represented an average of about 97% of the cannabinoids present in each sample (table 1). Other cannabinoids, Δ^9-THC in particular, as well as some CBN, were detected in small quantities in some samples (table 1).

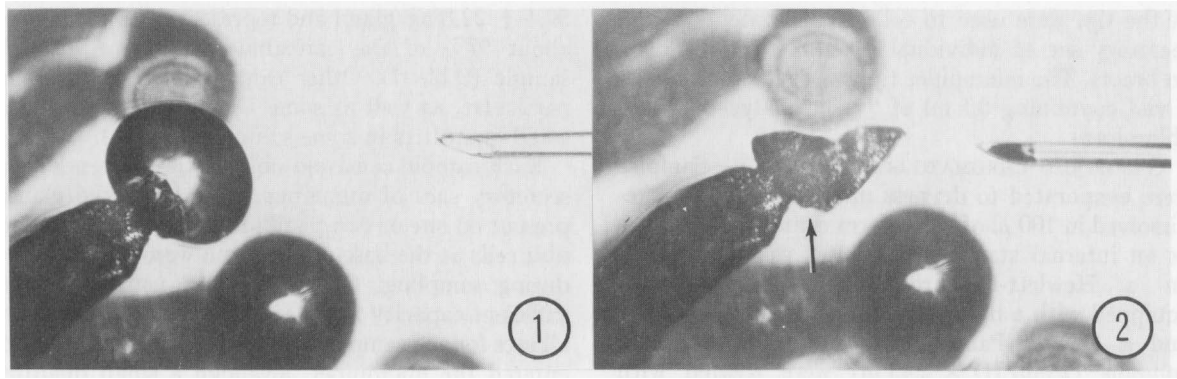
Each sample consisted only of the contents from secretory sacs of numerous capitate-stalked glands present on one or two pistillate bracts (table 1). The disk cells at the base of the gland were not damaged during sampling. One micropipet usually was of sufficient capacity to collect secretory products from all sacs for one sample. Most of the secretory product entered the micropipet, although a small quantity may have remained in the crumpled sac (figs. 1, 2). No secretory product appeared to be lost by exuding around the periphery of the micropipet.

A comparison of the cannabinoid profiles in this study with the ratios of cannabinoids for leaves and bracts of clone 87 (TURNER et al. 1977, 1980) indicated that the contents of the secretory sac were representative of the cannabinoid profile characteristic of this clone. The CBD content of the gland sac was also compared to the cannabinoid content reported for whole glands from this clone (TURNER et al. 1978). In those previous studies, samples of 20 intact gland heads were collected from the bract surface. The CBD was the only cannabinoid detected and averaged 35.3 ± 11.2 ng/gland, with a

TABLE 1

CANNABINOID CONTENTS IN SECRETORY SACS OF CAPITATE-STALKED GLANDULAR TRICHOMES FROM BRACTS OF CANNABIS

SAMPLE	No. GLANDS	BRACT LENGTH (mm)	ng CANNABINOIDS/GLAND			
			CBD	Δ^9-THC	CBN	Total
1	118	9	28.1	.6		28.7
2	103	9	27.9			27.9
3	200	8	37.6			37.6
4	207	8	34.5	.3		34.8
5	101	8	20.9	5.8		26.7
6	115	8.5	92.2			92.2
7	200	4	38.8			38.8
8	100	7	57.8	4.4		62.2
9	130	5	78.2			78.2
10	160	5	40.9			40.9
11	84	5.5	21.7	1.1		22.8
12	93	4	98.9	1.3		100.2
13	107	4.5, 5	77.8	1.9		79.7
14	100	4.5	68.4	1.3		69.7
15	115	4	76.5	1.3		77.8
16	110	4	89.3	5.6	7.9	102.8
17	103	5	85.0			85.0
18	97	5	76.4			76.4
19	109	6	70.5	2.1		72.6
20	103	4.5, 7	53.8			53.8
21	80	4.5	49.7	.8		50.5
22	198	6	48.0	.8	.8	49.6
23	193	4, 6	50.2	1.4	1.8	53.4
24	350	7, 8	59.5	.9	1.8	62.2
25	198	6.5	77.8	1.0	.2	79.0
26	175	6, 8	83.2	1.2	.1	84.5
Average values, ±SD			59.4±22.8			61.1±23.4



FIGS. 1, 2.—Capitate-stalked glands of *Cannabis*. Fig. 1, Micropipet near gland as observed through microscope on micromanipulator; gland is refractile in appearance; × 260. Fig. 2, Micropipet has collected contents of secretory sac of gland; note collapse of secretory sac and intact disk cells at base of gland (arrow); × 260.

range of 18.7 to 49.3 ng/gland (TURNER et al. 1978). The higher average quantity of CBD for each secretory sac sample determined in the present study, as contrasted to analyses of whole glands, may reflect differences in sample size. The average CBD content of a whole gland was calculated from five samples of 20 glands each, whereas the average cannabinoid content of a secretory sac was calculated from 26 samples consisting of 80–350 glands per sample.

The differences in the amounts of cannabinoids present in each of the 26 samples of gland secretory sacs may reflect procedural differences in sampling rather than the effects of external environmental influences. For example, the samples in table 1 are listed in chronological order, yet no specific temporal pattern for the cannabinoid content in the sac is evident during the collection period. However, we have shown that the cannabinoid content of glands can vary for glands positioned on vein areas as contrasted to nonvein areas (TURNER et al. 1978). In the current study, it was not possible to distinguish between these areas, and glands were collected randomly from the bract surface. In addition, the cannabinoid content varies with gland age (TURNER et al. 1977). In the present study the effect of age was minimized since glands of a characteristic appearance, indicative of their uniform age, were selected for sampling. However, bracts of different lengths were used as the source of glands (table 1); therefore, glands may have differed from each other slightly in stage of maturity. By comparison, the bracts used for the study of whole glands were all 9 mm long, and glands possibly were more uniformly mature.

Based on the data obtained in the present study, it appears that the majority of cannabinoids associated with capitate-stalked glands are present in the secretory sac. The presence of minor as well as major cannabinoids in both the secretory sac and the whole gland may aid in determining the site of cannabinoid synthesis. With the current knowledge of the abundance of cannabinoids in the secretory sac, we hypothesize that, if the disk cells are the site of cannabinoid synthesis, it then appears that the synthesized cannabinoids are secreted rather quickly into the secretory sac. HAMMOND and MAHLBERG (1978) reported that disk cells were unlike other epidermal cells in that they developed numerous plastids possessing a paracrystalline body which nearly filled the mature plastid. They also showed the secretory sac to be filled with a complex array of spherical membrane-like components. The unique organization of the gland disk cells and secretory sac, which is formed as a cavity within the wall, emphasizes the possibility that cannabinoid synthesis occurs in the gland. Further studies are in progress to determine the specific site of cannabinoid synthesis.

Other investigations showed that noncannabinoid compounds, which have been reported in *Cannabis* tissues (NOVOTNY et al. 1976; TURNER, BOEREN, and ELSOHLY 1976) may occur in glands or their secretory products. Mono- and sesquiterpenes have been reported among the "essential oils" and "resins" of this plant (HENDRIKS et al. 1975). Histochemical studies have shown that alkaloids, lipids, and products from cytochrome oxidase and lipase mediated reactions were present in glands (FURR and MAHLBERG 1981). TURNER et al. (1977) reported changes in the character of the sac content during aging in that the secretory materials become more cohesive and tacky and undergo changes in coloration. The changes in cannabinoid concentrations noted for the gland, therefore, may be paralleled by changes in other compounds in the gland and its secretory sac. To define the secretory process in *Cannabis*, it is essential to understand the chemical environment within the secretory sac. Studies of this nature are currently being done.

The complex secretory activities evident for the glands of *Cannabis* may be reflected in similar pat-

terns of secretion for glandular trichomes on other plants. The quantitative data obtained in this study provide a basis for further studies on the qualitative aspects of the secretory product, the site of cannabinoid synthesis, and a definition of the secretory process in *Cannabis*. More precise information in these areas is necessary to gain an understanding of the dynamics of secretory processes in glandular structures in general.

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