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Author(s): Ernest Small, H. D. Beckstead, Allan Chan

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# The Evolution of Cannabinoid Phenotypes in *Cannabis*<sup>1</sup>

ERNEST SMALL<sup>2</sup>, H. D. BECKSTEAD<sup>3</sup>, AND ALLAN CHAN<sup>2</sup>

## INTRODUCTION

The medical and public concern over the use of marihuana in our society strongly reflects the fact that *Cannabis sativa* is indeed an exceptionally important plant. Apart from its drug and fibre products, it is economically important from yet another aspect: that of being a serious weed in some countries, necessitating costly eradication. Botanists are participating in the accelerated research on *Cannabis* which began roughly five years ago, and several botanical programs are in progress around the world. The research described here may duplicate to some extent the findings of other researchers. We believe, however, that verification of research findings is necessary for a plant as important as *Cannabis*, particularly as some of our findings either contradict earlier generalizations or have not yet been recorded in the literature.

In Canada, one research project on *Cannabis* is being conducted jointly between the Plant Research Institute of the Canada Department of Agriculture, and the Health Protection Branch of the Canada Department of National Health and Welfare. The senior author is directly responsible for the botanical aspects, and the second author for chemical analysis.

Although several aspects are being investigated, the research reported focuses mainly on the question of natural variation in *Cannabis sativa*, with particular emphasis on the cannabinoids — the class of compounds responsible for the psychoactive properties of cannabis drugs. A

historical and biological context for the major chemical variants of *Cannabis sativa* which can be discerned will be presented.

A brief review of several aspects bearing on natural variation in *Cannabis* may be useful before presenting some of our results.

## MALE AND FEMALE PLANTS

The male plants of *Cannabis sativa* are usually more slender than the females and come into flower earlier. However, the flowering periods overlap sufficiently to ensure fertilization. *Cannabis* is wind-pollinated, and a large male plant may shed a million pollen grains. The males die soon after shedding their pollen. Female plants are killed by frost, but in greenhouses we have grown them as perennials. These annual plants have been known to exceed 25 feet in height and 4 inches in stem diameter.

## STEMS

*Cannabis* has been described as a triple-purpose plant, furnishing three major products of interest to man. It is from the stems that we got the first useful product, the fibres, which are still valuable in commerce (Fig. 1). In fact, *Cannabis* is the oldest known cultivated fibre plant. The fibres, which are located in the bast or bark, are obtained by retting the stems. Although hemp fibres have become less important due to recently developed synthetics, *Cannabis* is still widely cultivated in north-eastern Asia and Europe as a fibre crop. There also is some interest, currently, in the possibility of using hemp as a source of paper pulp. Many varieties of *Cannabis* have been bred for fibre content and, in particular, monoecious varieties have been created in order to

<sup>1</sup>Contribution No. 960, Plant Research Institute.

<sup>2</sup>Canada Department of Agriculture, Research Branch, Ottawa, Ontario.

<sup>3</sup>Health Protection Branch, Department of National Health and Welfare, Ottawa, Ontario.

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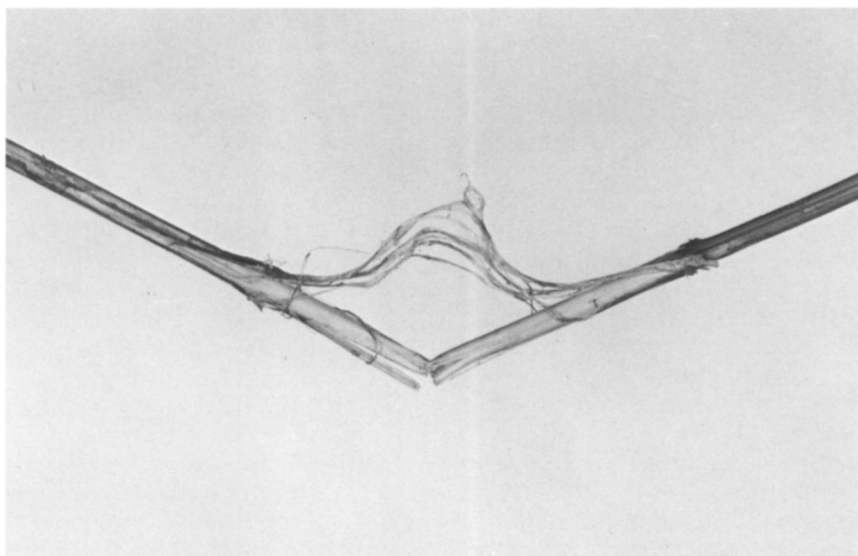


Fig. 1. Retted stem of *Cannabis*, showing fibres displaced to one side.



Fig. 2. Fruit of *Cannabis*. Enclosing bract is present on younger "seeds".

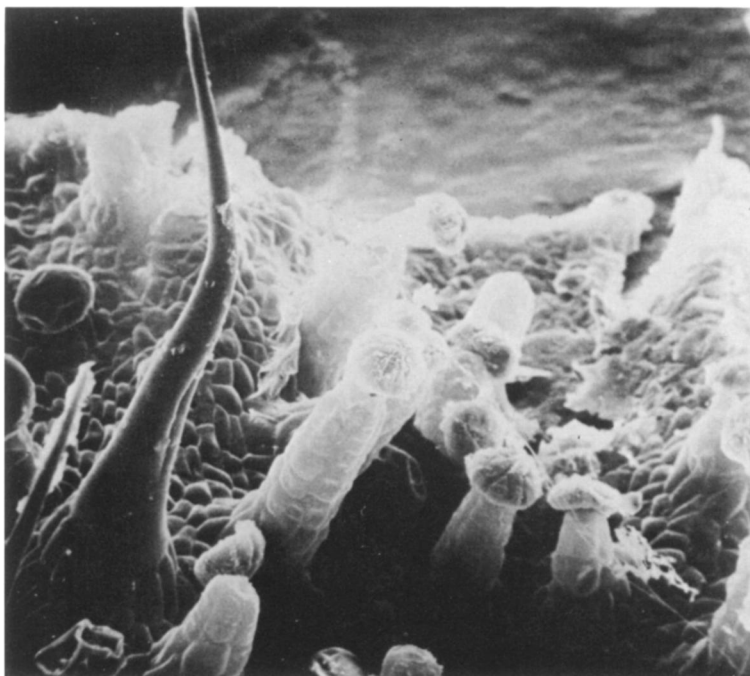


Fig. 3. Scanning electron microscope of upper surface of a bract which encloses the fruit in *Cannabis*. Note capped secretory glands, and cystolith hair.

overcome the troublesome sexual dimorphism (Feaster, 1956). Because male plants have relatively long internodes, they yield a better quality of fibre than females.

#### SEEDS

A second agricultural product of *Cannabis* is the fruit (Fig. 2). The fruits are achenes, but are commonly called seeds. They contain an oil, which is very much like linseed oil, and is used in paints and in soap-making. And, after the oil is extracted from the seeds, the remaining oil-cake may be fed to cattle. In Europe, the so-called "seeds" are often roasted and eaten by man. Bird feed mixtures often contain *Cannabis* seeds. The seeds are also valuable as a growth medium for many water molds, and the antibiotic they contain, which is active against gram-positive bacteria, is receiving considerable medical interest. We have analyzed the oil content of several hundred

acquisitions of *Cannabis* seeds, and find little difference between varieties of *Cannabis* used for oil and fibre, and varieties used for drug purposes. One variety known as "oleifera" contains about 40% oil and is widely grown in Russia because of the high oil content of the seeds. Most varieties of *Cannabis* contain about 30% oil in their seeds. A good oil variety, rather than containing an especially high percentage of oil, would be one which produces high yields of seeds.

#### EPIDERMAL GLANDS

The third substance for which *Cannabis* is known and the product of primary concern is a drug which is secreted in the form of a resinous exudate by epidermal glands. The small glistening glands are especially abundant on the upper surfaces of the leaves. It is known that active materials are also synthesized in internal tissues of the plant, in single-celled laticifers (Fujita et al., 1967). However, the

bulk of the resinous material responsible for the psychoactive effects of marihuana and hashish is synthesized in the epidermal glands. A speculative and teleological explanation of the purpose of the resinous secretion is that the resin is a kind of varnish, intended to protect the leaves against the sun. In view of our inability to explain the adaptive purpose, if indeed there is one, of many natural plant products, we need not be unduly concerned about what the resin does for the plant. On the leaves, the glands are usually hemispherical. In the reproductive parts of the plants, however, the glands are often more elaborate, and more numerous.

The bract which covers the developing seed is especially well-endowed with secretory glands. A scanning electron micrograph of the surface of such a bract shows that the multicellular glands are stalked and capped (Fig. 3). The cap is rather loosely attached, and often gets knocked off. Additionally, a cystolith hair is shown. This unicellular hair, which contains an internal concentration of calcium carbonate, is considered diagnostic for forensic identification of cannabis drugs.

#### HISTORICAL DISTRIBUTION

From the fact that *Cannabis* has been used by man for different purposes, it is clear that an understanding of the evolution of different variants or kinds of *Cannabis* necessitates consideration of the historical association between this plant and man. As will become apparent later, the importations of *Cannabis* by man are a key to an understanding of the present distribution of chemical varieties of *Cannabis*. The historical ethology of *Cannabis* has been extensively described (Bouquet, 1950; Schultes, 1970), and only a brief background will be given here.

Although it is apparent that *Cannabis* once had a comparatively restricted distribution area in Asia, it is very difficult on the basis of present evidence to establish the original natural distribution before the range was greatly extended by man. Many authors have suggested that the

native range included temperate parts of Asia, possibly parts of Iran, southern Siberia, the Kirghiz Desert, India, and the Himalayas.

Certainly *Cannabis* was cultivated in very early times. Hemp cloth, estimated at 6,000 years of age, has turned up in Europe. However, written accounts of *Cannabis* are more recent. As there are no references to *Cannabis* in hieroglyphic texts or in the scriptures, the plant appears to have been unknown to the ancient Egyptians and Hebrews. The first written reference appears to be a Chinese work, dated at 2737 B.C., in which the pharmacological properties of *Cannabis* were mentioned. The Greeks and Romans knew of the fibre content of hemp, but were apparently unaware of its drug properties. However, the drug properties of *Cannabis* were well known in India before Christ, as recorded in early religious writings. *Cannabis* was used as a drug in Iran, six centuries before the Christian era. During the first thousand years of the Christian era, the drug use of *Cannabis* spread to various Mediterranean circle countries, and to Africa. Until the sixteenth century, *Cannabis* was known mainly for its fibre in Europe. Then the age of exploration began, and cannabis drugs were commonly brought back to Europe. Nevertheless, the use of cannabis drugs never became very popular in Europe, and *Cannabis* continued to be mainly known for its fibre qualities. From Europe, *Cannabis* was brought to Chile about 1545 (Dewey, 1914; Kalant, 1968), and was introduced into the U.S. into New England in the Puritan settlements about 1632. The first recorded cultivation of hemp in North America, however, was in Port Royal, Acadia, Nova Scotia, in 1606 (Lescarbot, 1609). Because of the agricultural and drug importance of *Cannabis*, the plant has become distributed over most of the world. With frequent escapes from cultivation, *Cannabis* has become naturalized or "wild" in many countries and has posed serious weed problems.

One of the principal aims of our program was to examine the structure of the world variation pattern of *Cannabis*, with



Fig. 4. A portion of the *Cannabis* plantation in Ottawa.

particular emphasis on natural variation of the psychoactive materials. We set out to acquire as many seed stocks of *Cannabis* as possible, particularly attempting to sample all possible sources of variation. We contacted seed exchange institutions, police units, botanical gardens, agricultural research stations, research institutes and individuals working on *Cannabis* around the world. Through the generous assistance of several hundred individuals, we assembled 350 different viable seed stocks from about 50 countries. These were grown in a three-acre plot (Fig. 4) in 1971, in Canada's Central Experimental Farm, located in Ottawa.

#### CULTURAL METHODS

Each strain was planted in two random 25-foot rows. The growing season is relatively short in Ottawa, and as *Cannabis* seedlings are quite susceptible to frost, we did not sow until the beginning of June. One-half acre of this site was devoted to growing a standard strain of *Cannabis* for the production of marihuana to supply authorized Canadian researchers. Through the generosity of the National Institute of Mental Health (NIMH), we obtained and grew the same standard strain used to grow bulk research material for NIMH by the University of Mississippi.

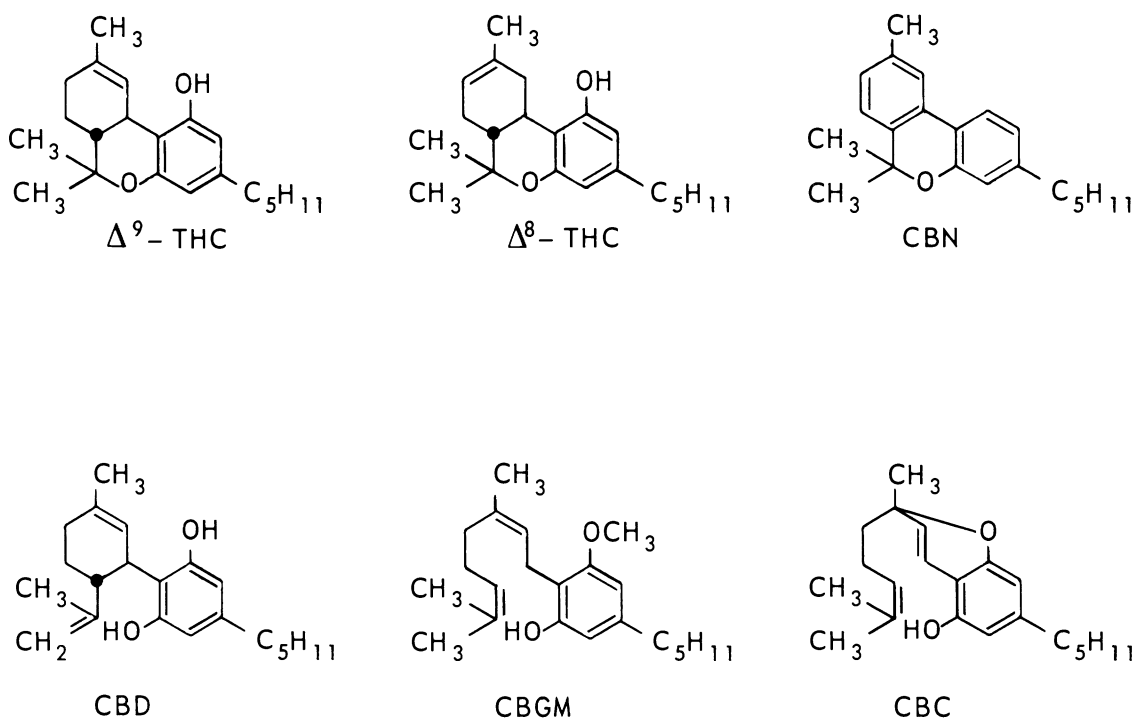


Fig. 5. Chemical diagrams of cannabinoids mentioned in text.

By late August, toward the end of the growing season, about 70% of our stocks had come into flower. However, many strains failed to reach the flowering stage and are, therefore, not adapted to completing their life cycle in a north-temperate climate equivalent to Ottawa. The standard strain we used to grow bulk material barely matured before frost. We will comment later on the comparative performance of that strain in Ottawa and in Mississippi.

#### PROCESSING METHODS

We used rather simple harvesting equipment such as axes and heavy cutters to harvest the plants. Plants were collected for analysis as they matured — the males while they were shedding pollen, and the females several weeks later, when the first mature seeds were present. Some varieties came into flower only six weeks after they were planted. However, as we have commented, several strains did not come into

flower at all, and these were harvested in September when there was danger of frost.

A tobacco barn was used to dry our material. Movable slats on the side of the barn controlled ventilation. A hay-drying oil heater was put into use during cool and wet periods. Drying indoors is much preferable to drying outdoors, as dew and rain appear to lower the quality of the product.

For each plant analyzed, we prepared a sample of "manicured" marihuana. This was accomplished by sieving the plants through a fine mesh wire screen. This had the effect of screening out most of the seeds, larger twigs, and coarse material, while allowing the shredded leaves and floral parts to fall through. For each of the 350 strains, three male plants and three female plants were examined. For plants which failed to flower and for a number of monoecious strains, six plants were also examined.

Although small quantities of alkaloids have been found in *Cannabis* (Klein and Rapoport, 1971), it is generally believed that the psychoactive effects of cannabis drugs are due to that class of compounds known as the cannabinoids or cannabinols (Mechoulam, 1970). Six of the chief cannabinoids are shown in Fig. 5. The two tetrahydrocannabinol compounds shown are believed to be psychotomimetic.  $\Delta^8$ -THC usually occurs in very limited quantity compared to  $\Delta^9$ -THC (adopting formal pyran nomenclature for these compounds), and the latter is thought to be chiefly responsible for the psychotomimetic effects of cannabis drugs. The other compounds are not psychotomimetic, although cannabidiol (CBD) in carboxylated form is thought to have sedative properties (Farnsworth, 1969). Cannabinol (CBN) is believed to be an oxidation product, probably from THC, which appears on aging cannabis preparations (Ohlsson et al., 1971). We agree with this interpretation as we found almost no CBN in any of our freshly harvested samples. Homologues of CBD, THC and CBN, in which the side-chain is  $C_3H_7$  instead of  $C_5H_{11}$ , have also been identified (Vollner et al., 1969; Merkus, 1971; Vree et al., 1972) and the propyl homologue of THC, which may occur in appreciative quantity, has been found to have biological activity (Gill et al., 1970). Homologues with methyl side-chains have also been discovered in very limited amounts (Vree et al., 1972). These compounds exist substantially in a carboxylated state in the living plant and in freshly harvested material. When a carboxyl group is appended to THC, the resulting molecule is believed to be non-psychotomimetic. This is irrelevant if smoking is the method of consumption, as the heat of smoking results in decarboxylation.

#### CHEMICAL ANALYSIS

We analyzed all our samples by gas-liquid chromatography using methods which have been described previously (Beckstead and French, 1971). A typical

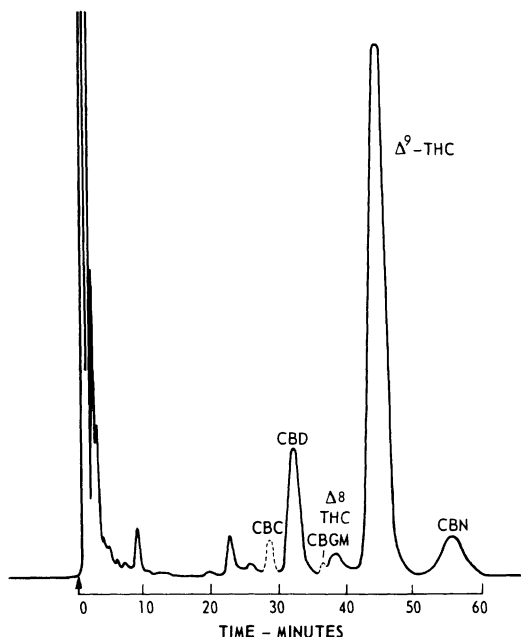


Fig. 6. A gas-liquid chromatogram of an n-hexane extract of marihuana (type I) prepared from a strain used to grow bulk material. The locations of CBC and CBGM in our GLC system are indicated by broken lines.

chromatogram of a sample from the standard strain we used to grow bulk material shows that this material is quite high in  $\Delta^9$ -THC and quite low in CBD (Fig. 6).

#### THE CANNABINOID PHENOTYPES

Before we began our survey of the cannabinoid characteristics of various strains, it was known that at least two classes of plants are to be found (Fetterman et al., 1971). In one type, there are large amounts of  $\Delta^9$ -THC and small amounts of CBD. In contrast, other strains contain the reverse ratio: abundant CBD and very little THC, and because they contain so little THC, such strains are not suitable for the preparation of cannabis drugs.

Several investigators (De Faubert Maunder, 1970; Korte et al., 1965; Turner and Hadley, 1973) have reported that in some samples of plants with high amounts of THC, no CBD can be detected. Turner



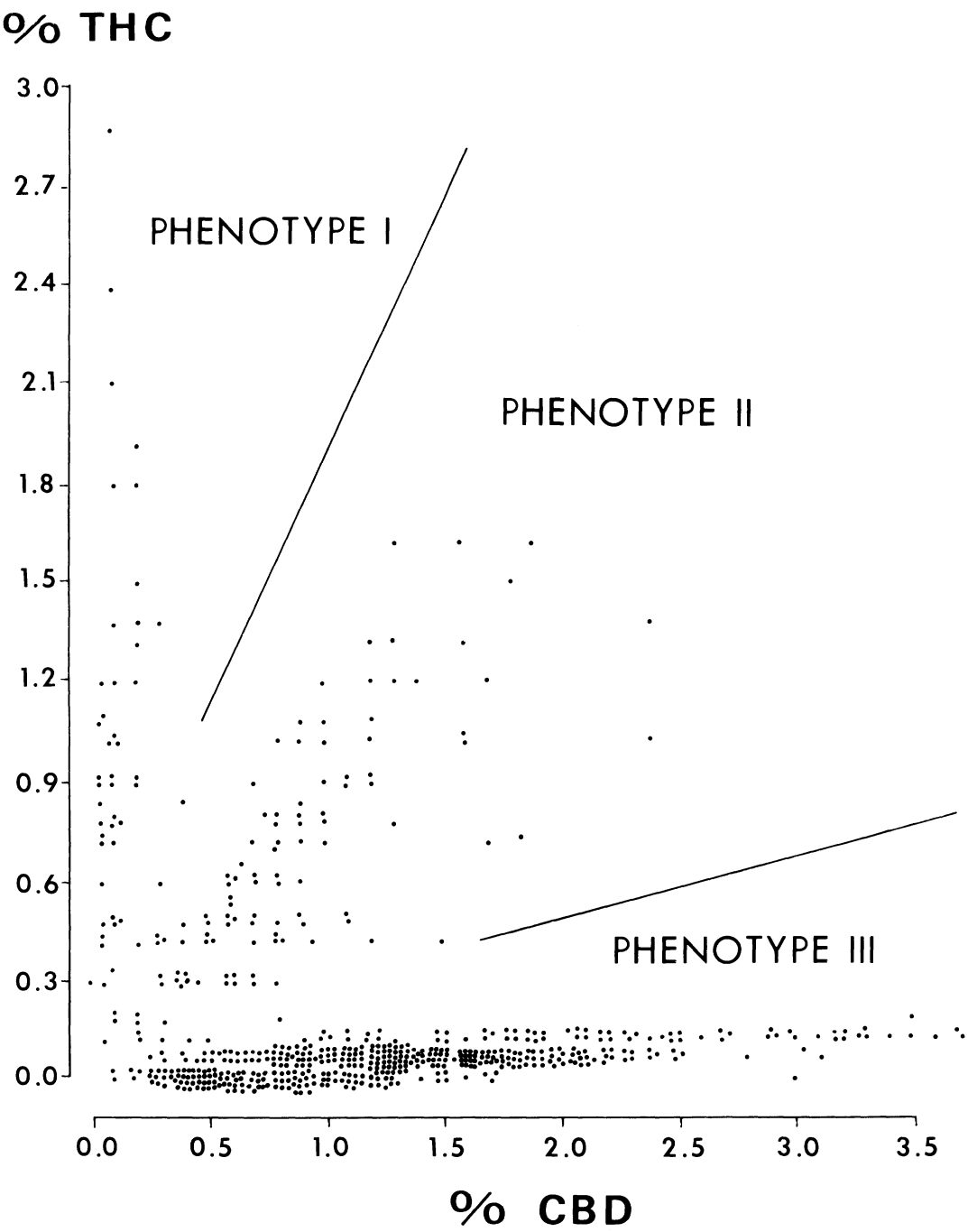


Fig. 7. Plot of THC content against CBD content for female plants. A few individuals for populations classified as phenotype II fall into range of phenotype III.

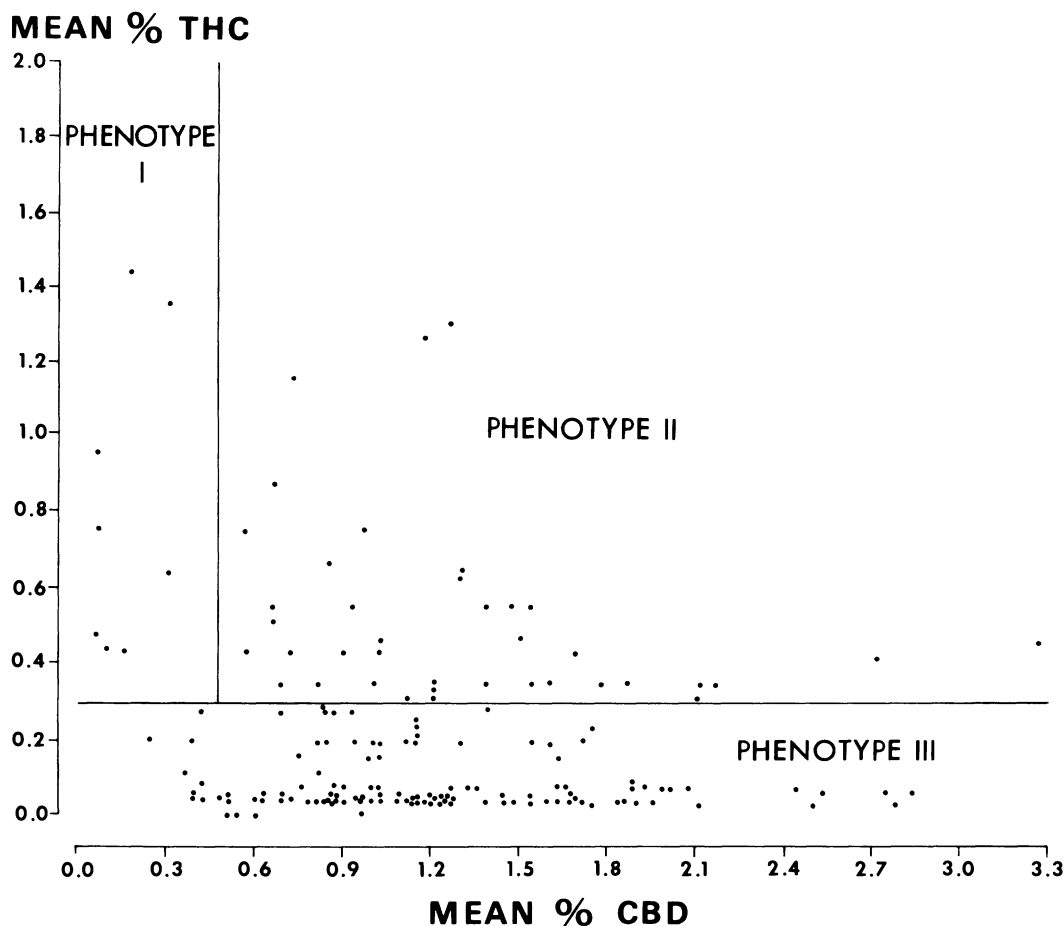


Fig. 8. Plot of mean THC content against mean CBD content for populations of female plants.

and Hadley state that in most GLC systems, CBD cannot be distinguished from two other cannabinoids, cannabichromene (CBC), and cannabivarin (the propyl side-chain homologue of CBN). It is possible that in the past, reports of small amounts of CBD were in fact based on the presence of CBC.

Our main goal was to verify the existence of the two basic categories of plant, one rich in  $\Delta^9$ -THC, and the other very low in this constituent. As a result of our studies, we found two other common cannabinoid patterns, and we noted that in two of the four cannabinoid phenotypes which we propose be recognized,

there is a pronounced difference in cannabinoid content of the male and female plants. We shall now present evidence bearing on these phenotypes. For the moment we will discuss three contrasting cannabinoid phenotypes which we refer to as types I, II, and III, and will describe a type IV later.

A scatter diagram shows THC content plotted against CBD content for all of the plants we examined which came into flower as females (Fig. 7). The individuals fall into three main clusters. The cluster falling close to the ordinate consists of plants in which the resin is composed primarily of THC. Plants of this type are

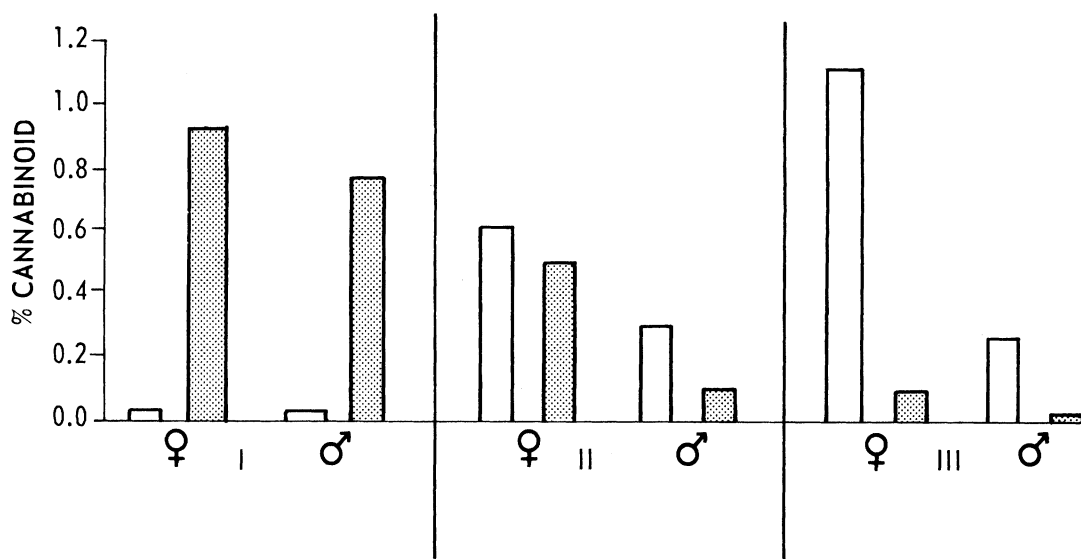


Fig. 9. Diagrammatic representation of common patterns of cannabinoid association. Numerals refer to phenotype designations. Open bars = CBD; stippled bars = THC. Height of bars is proportional to mean values for the strains examined which conformed to the "type".

designated phenotype I. These plants are obviously suitable for the preparation of cannabis drugs. A contrasting cluster, falling close to the abscissa, is designated as phenotype III. In these plants, the resin is composed primarily of CBD, and accordingly such plants are not suitable for the preparation of *Cannabis* drugs. The cluster of points at an angle of  $45^\circ$  between the ordinate and the abscissa represents plants containing intermediate amounts of THC and CBD. In general, these contain sufficient THC to be considered capable of producing potent drug preparations. Actually, the intermediate type, designated here as phenotype II, is not sharply demarcated from phenotype III. For several of the populations which we classified as phenotype II, only some of the plants contained intermediate amounts of THC and CBD, while the resin of other plants of the population was composed primarily of CBD.

The scatter diagram just presented exaggerates the distinctiveness of the phenotypes which are more accurately shown in Fig. 8. The results for the means of the populations — usually the means of

three female plants — are plotted in Fig. 8. The types tend to merge somewhat more than in the diagram for individuals, and their delimitation is arbitrary. We have defined phenotype I as consisting of populations with more than 0.3% THC and less than 0.5% CBD. Phenotype III consists of populations with less than 0.3% THC. And the intermediate phenotype II consists of population averaging more than 0.3% THC but also more than 0.5% CBD. Of course these absolute figures are only relevant to plants grown in a climate like Ottawa, and may not apply to plants grown in other climates. Most of the strains which failed to flower in Ottawa possessed resin which was composed primarily of THC and belong to this phenotype.

A plot of THC against CBD for the males of the corresponding populations would be similar in some respects to the diagram for females, but would be more difficult to interpret because in almost all strains of phenotypes II and III, the male plants have a much smaller resin content, although the proportions of THC and CBD are roughly similar to the females.

The three phenotypes discussed to this point are shown in histogram fashion, with the situation shown separately for males and females in Fig. 9. The amount of the psychotomimetic cannabinoid, THC, is shown by a stippled bar, and the amount of the non-psychotomimetic cannabinoid, CBD, is shown by an open bar. The height of the bars represents the averages we found for populations conforming to the phenotype. In type I, both male and female plants have high amounts of THC, and low amounts of CBD. Such populations almost always originated from countries south of latitude 30°N. These often flowered late or completely failed to flower, indicating such strains are adapted to completing their life cycle in a longer growing season than possible in Ottawa. Phenotypes II and III contrasted with phenotype I in four respects. Firstly, female plants were always very considerably higher in cannabinoid content than the males, in contrast with phenotype I where we found only a slight discrepancy in cannabinoid content of males and females. Secondly, in contrast to phenotype I, types II and III usually originated from countries north of latitude 30°N. Thirdly, types II and III almost always flowered fairly early in the summer, indicating they are adapted to completing their life cycle in a north temperate climate. And finally, types II and III almost always contain large amounts of CBD. The only difference between types II and III lies in the higher amounts of THC in type II. Hence, type II is quite potent, or at least the females of type II are quite potent. Type II plants were often represented by varieties of *Cannabis* grown for fibre and, hence, the often advanced opinion that fibre varieties of *Cannabis* do not have drug properties is not always correct.

In addition to these three chemical phenotypes, we discovered another widespread geographical phenotype in northeastern Asia (including Japan, Korea, and the Peking area of China) which we refer to as type IV. Plants from northeastern Asia consistently possessed trace amounts (about 0.05% of the weight of the manicured marijuana prepared from the

plants) of a cannabinoid having the same retention time as cannabigerol monomethyl ether (CBGM). Yamauchi et al. (1968) reported that CBGM was present in a Japanese variety of *Cannabis* known as "Minamioshihara", which was among our samples conforming to type IV. We were able to distinguish CBGM from  $\Delta^8$ -THC only with difficulty, since the retention times by GLC are very similar. Turner (pers. comm.) has pointed out that the retention time of hexahydrocannabinol also is similar to that of CBGM. One of our goals is to correlate morphological features of strains of *Cannabis* with their chemistry, and we are currently examining the morphology of our strains. Although our results are quite preliminary, it seems that this northeastern Asian variant is morphologically distinctive, having, for example, exceptionally tall plants.

#### RELATIVE POTENCY OF MALES AND FEMALES

Our work bears on a question which has been controversial for a very long time: the relative potency of males and females. Although females are larger than males, and so produce more material per plant, the important point is their comparative performance on a weight basis. For a long period it was believed that males never have drug properties. This belief appears to have been stimulated by the often noted practice in India and other Far-East countries, of roguing male plants out of the field as soon as they can be recognized. This removal of the males frequently represents an attempt to prevent pollination, as this is said to increase the potency of the plants. This may be so; if the plant's energy is not directed into producing seeds, the food reserves may be converted to the production of more floral tissue, which is higher in resin content. In any case, males are often discarded in the belief that they are not potent. Recently, several investigators have examined single strains of *Cannabis* and concluded males and females are equal in potency. Our comprehensive survey indicates that the relative potency of

males and females depends on the particular strain. In type I strains, males and females are approximately equally potent, but in phenotypes II and III, males are less potent than females. Of course, type III strains are so low in THC that even the females are not suitable for the preparation of cannabis drugs. However, with type II strains, potent drugs could be made from the females, but not from the males.

While we are comparing males and females, we might mention that for the type I strain we used to grow bulk material the ratio of THC to CBD differed for the sexes. Because male flowers had relatively higher amounts of CBD, mature males had a smaller ratio of THC/CBD than did females. As can be appreciated from Fig. 9, however, the difference is slight.

#### DISTRIBUTION OF CHEMICAL PHENOTYPES

There is a strong correlation between the distribution of the phenotypes and the historical uses of *Cannabis* for drug and for non-drug purposes described earlier. Latitude 30°N constitutes an important dividing line. Type I plants usually originate south of this parallel. Generally the growing seasons in countries south of latitude 30°N, in which *Cannabis* is cultivated, are relatively long. This would explain the failure of most phenotype I strains to flower in Ottawa. As we have remarked, such strains have very high amounts of THC. Plants in such areas as India and Africa, historically, have been selected for drug content, and it is not unexpected that strains originating from these areas are high in THC. Although there seems to have been less selection for males than for females, it appears that selection has resulted in both sexes having high THC contents. Despite the fact that *Cannabis* was originally imported into the New World, both north and south of 30°N, for fibre purposes, a considerable number of type I strains were found in Central and South America. This would indicate that a relatively recent importation of drug strains of *Cannabis* took

place. Phenotype III strains usually originate from countries north of latitude 30°N, although occasional strains in which the resin is mostly composed of CBD also originate from countries south of latitude 30°N. This is clearly explicable in terms of the historical use of *Cannabis* in northern countries for fibre and for oil rather than for drug purposes. And as the growing season in such north temperate countries is generally comparatively short, it is hardly surprising that phenotype III strains are relatively early in maturation. The existence of a category intermediate between phenotypes I and III, phenotype II, is more difficult to explain. It may be that intermediate populations simply represent natural variation. However, we suspect that many such intermediate strains originated by hybridization between phenotype I and phenotype III strains. Drug strains and non-drug strains have often been imported by hemp breeders and dealers in cannabis drugs, and it would be surprising if frequent hybridization did not take place.

We found that most phenotype II strains bore closer resemblances to phenotype III strains than to phenotype I. In other words, intermediates were more common among the high CBD strains of the north than among the high THC strains of the south. However, it should be noted that *Cannabis* is frequently grown for fibre in countries south of latitude 30°N, as well as for drug, and such so-called fibre strains usually were intermediate in cannabinoid composition, and so assignable to phenotype II.

#### TAXONOMIC PROBLEMS IN *CANNABIS*

Now the delimitation of four chemical phenotypes raises the question of how many taxonomic groups should be formally recognized, and at what level these should be recognized. Anyone who examines the literature dealing with *Cannabis* quickly realizes that many taxonomic names, especially specific names, have been proposed. As no comprehensive study of variation of *Cannabis* has ever

been published, we have set out to clarify its taxonomy. There are two generally accepted bases for formal taxonomic delimitation: morphological distinction and interfertility. Our morphological studies are still in a preliminary stage, and we are not yet in a position to say how many units should be recognized on morphological grounds. However, we have examined the possibility of genetic barriers to breeding, and in particular for the possibility of chromosomal barriers to breeding. We hybridized 38 populations, representing the four chemical phenotypes, and a variety of so-called species of *Cannabis* with each other and examined the hybrids for the degree of chromosomal homology between the parents. All of our hybrids showed no chromosomal aberrations, perfectly normal meiosis, and completely stainable pollen (Small, 1972).

In addition, we found it very easy to produce hybrids by cross-pollination, and the hybrids were exceptionally vigorous. This means that there are apparently no breeding barriers within *Cannabis*, and for taxonomists who believe that species should not be recognized unless breeding barriers have developed, it means species should not be recognized in *Cannabis*.

All of our populations had the diploid number of chromosomes,  $2n = 20$ . Polyploids have been generated for agricultural purposes, and because polyploids and diploids are usually intersterile, if a polyploid race became widely established geographically, there might be a basis for giving it taxonomic recognition. Although we have noted occasional vague references to geographically-established polyploid races in *Cannabis*, we can find no authoritative description of these.

Incidentally, we examined most of our hybrid combinations for their cannabinoid content. Usually the ratio of THC to CBD in the hybrids was approximately intermediate between the parents. However, there was also occasionally a small but statistically significant deviation toward one of the parents—not necessarily the one with the higher or the lower ratio of THC to CBD.

Finally, we would like to comment on the important issue of the influence of the environment on cannabinoid production. We are currently studying the effects of several environmental factors on cannabinoid development in *Cannabis*. We have, for example, found that plants grown in sand, fertilized weekly, have higher contents of THC than plants grown in loam, but otherwise treated similarly. We are unable at present to identify the edaphic factor responsible. We have grown plants under extreme deficiency of nitrogen, phosphorus and potassium, and although the plants were highly dwarfed, their THC content was roughly comparable to that of control plants. We also have limited data indicating that shading decreases THC production. However, on the whole, our preliminary results suggest that environmental effects are relatively slight. The question of the environment is obviously relevant to a consideration of the cannabinoid phenotypes we have proposed, for one would like some assurance that the categories we have recognized on the basis of growing 350 stocks in Ottawa would be the same if the stocks were grown in a more southern climate. One disquieting observation relates to the copious resin exudation often described for drug strains grown in very hot climates. In Ottawa, we observed comparatively little resin exudation, and indeed the total cannabinoid content of the drug strains was about the same as it was for the non-drug strains, that is, about 2% on a dry weight manicured material basis. As noted earlier, we grew the same strain in Ottawa that is grown in Mississippi for bulk production. The yield in Ottawa was exceptionally good: about 900 pounds of manicured marihuana from half an acre. Our carefully cultivated plants produced material of about 1½% THC content. We understand from Dr. Carleton Turner that carefully harvested plants of about the same age in Mississippi might contain 3% THC. This is perhaps an unfair comparison, since our plants were never drought stressed while we understand Mississippi

plants often lose their lower, less potent leaves because of drought, thereby increasing the net potency of the plant. It is our opinion that, although different climates might yield different quantities of resin from *Cannabis*, the essential ratios of the cannabinoids which define our four cannabinoid phenotypes would remain constant, and so our phenotypes could be recognized anywhere. We look forward, in any event, to clarification of the role of the environment in cannabinoid production

#### LITERATURE CITED

- Beckstead, H. D. and W. N. French. 1971. Some analytical methods for drugs subject to abuse. Canadian Department of National Health and Welfare. Ottawa. 307 pp.
- Bouquet, R. J. 1950. *Cannabis*. Bull. Narc. 2: 14-30.
- De Faubert Maunder, M. J. 1970. A comparative evaluation of the  $\Delta^9$ -tetrahydrocannabinol content of *Cannabis* plants. J.A. P.A. 8: 42-47.
- Dewey, L. H. 1914. Yearbook of the U.S. Department of Agriculture, 1913. 283-346.
- Farnsworth, N. R. 1969. Pharmacognosy and chemistry of *Cannabis sativa*. J. Am. Pharm. Ass. NS9: 410-440.
- Feaster, C. V. 1956. Monoecious hemp breeding in the United States. Fibres (Engineering and Chem.) 17: 339-340.
- Fetterman, P. S., E. S. Keith, C. W. Waller, O. Guerrero, N. J. Doorenbos, and M. W. Quimby. 1971. Mississippi grown *Cannabis sativa* L. A preliminary observation on the chemical definition of phenotype and variations in THC content versus age, sex, and plant part. J. Pharm. Sci. 60: 1246-1249.
- Fujita, M., H. Shimomura, E. Kuriyama, M. Shigehiro, and M. Akusu. 1967. Studies on cannabis (2). Examination of the narcotic and its related components in hems, crude drugs, and plant organs by gas-liquid chromatography and thin-layer chromatography. Jap. J. Pharmacog. 21: 57-64.
- Gill, E. W., W. D. M. Paton, and R. G. Pertwee. 1970. Preliminary experiments on the chemistry and pharmacology of *Cannabis*. Nature 228: 134-136.
- Kalant, O. J. 1968. An interim guide to the *Cannabis* (marijuana) literature. Addiction Research Foundation. Toronto. 39 pp.
- Klein, F. K. and H. A. Rapoport. 1971. *Cannabis* alkaloids. Nature 232: 258-259.
- Korte, F., H. Sieper, and S. Tira. 1965. New results on hashish-specific constituents. Bull. Narc. 17: 35-43.
- Lescarbot, M. 1609. The history of New France. English translation by W. L. Grant. Toronto. The Champlain Society, 1907-14, 3 vols.
- Mechoulam, R. 1970. Marihuana chemistry. Science 168: 1159-1166.
- Merkus, F. W. H. M. 1971. Cannabivarin and tetrahydrocannabivarin, two new constituents of hashish. Nature 232: 579-580.
- Ohlsson, A. C., I. Abou-chaar, S. Agurell, I. M. Nilsson, K. Olofsson, and F. Sandberg. 1971. Cannabinoid constituents of male and female *Cannabis sativa*. Bull. Narc. 23: 29-32.
- Quimby, M. W., N. J. Doorenbos, C. E. Turner and A. Masoud. 1973. Mississippi-Grown Marihuana — *Cannabis sativa*: Its Cultivation and Morphological Variations. Econ. Bot. 27: 117-127.
- Schultes, R. E. 1970. Random thoughts and queries on the botany of *Cannabis*. In: Joyce, C. R. B. and S. H. Curry, The Botany and Chemistry of *Cannabis*. J. & A. Churchill. London, England. 11-38.
- Small, E. 1972. Infertility and chromosomal uniformity in *Cannabis*. Can. J. Bot. 50: 1947-1949.
- Turner, C. E. and K. H. Hadley. 1973. Constituents of *Cannabis sativa* L. II: Absence of cannabidiol in an African variant. J. Pharm. Sci. In press.
- Vollner, L., D. Bieniek, and F. Korte. 1969. Haschisch XX. Tetrahedron Lett. No. 3, 145-147.
- Vree, T. B., D. D. Breimer, C. A. M. van Ginneken, and J. M. van Rossum. 1972. Identification in hashish of tetrahydrocannabinol, cannabidiol and cannabinol analogues with a methyl side-chain. J. Pharm. Pharmac. 24: 7-12.
- Yamauchi, T., Y. Shoyama, Y. Matsuo, and T. Nishioka. 1968. Cannabigerol monomethyl ether, a new component of hemp. Chem. Pharm. Bull. 16: 1164-1165.