BULLETIN ON NARCOTICS

1983

UNITED NATIONS

DIVISION OF NARCOTIC DRUGS
Vienna

BULLETIN ON NARCOTICS

Vol. XXXV, No. 1
January – March 1983

UNITED NATIONS
New York, 1983

UNITED NATIONS PUBLICATION
00600P

NOTE

The *Bulletin on Narcotics* seeks to cover all aspects of national and international drugs control; the work of the international bodies responsible in this field; and developments concerning the research, assessment and prevention of drug abuse and the treatment, rehabilitation and social reintegration of persons dependent on drugs. Particular attention is paid to articles presenting national experience in drug abuse control which may be of interest and value to other countries.

The *Bulletin on Narcotics* is published quarterly in English, French and Spanish. Selected articles are subsequently published in Russian, and a summary of each volume is issued in Chinese every year.

Articles for publication in the *Bulletin* should be addressed to the *Bulletin on Narcotics*, Division of Narcotic Drugs, United Nations, Vienna International Centre, P.O. Box 500, A-1400 Vienna, Austria, accompanied by an abstract of approximately 200 words and a short *curriculum vitae* of the authors. They should be submitted in original and two copies. Illustrations consisting of all material that cannot be set in type, such as photographs, line drawings, graphs, charts and tracings may be included with the manuscript. Tables should be self-explanatory and should supplement, not duplicate the text. Each table must have a title. References should follow numerical order. The bibliography should be presented in alphabetical order.

A transmittal letter should designate one author as correspondent and include his complete address and telephone number.

Opinions expressed in articles published in the *Bulletin on Narcotics* are the responsibility of the respective authors and do not necessarily reflect those of the United Nations. The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the Secretariat of the United Nations concerning the legal status of any country, territory, city or area, or its authorities, or concerning the delimitation of any frontiers or boundaries.

Manuscripts not accepted are returned to the authors; however, the United Nations cannot be held responsible for loss.

Requests for subscription ($US 20.00 per annum) and all correspondence relating thereto should be addressed as follows:

for North America, South America, Asia and Oceania:
United Nations Sales Section
New York, N. Y. 10017
United States of America

for Europe, Africa and the Middle East:
United Nations Sales Section
CH-1211 Geneva 10
Switzerland

CONTENTS

The physical and chemical features of *Cannabis* plants grown in the United Kingdom of Great Britain and Northern Ireland from seeds of known origin – Part II: second generation studies

P. B. BAKER, T. A. GOUGH and B. J. TAYLOR
Laboratory of the Government Chemist, Cornwall House, London, United Kingdom of Great Britain and Northern Ireland

ABSTRACT

A second generation of *Cannabis* plants has been grown from seeds produced by first generation plants which were raised in the United Kingdom of Great Britain and Northern Ireland from seeds taken from imported cannabis of known geographical origin and chemistry. The gross physical appearance of the second generation plants, in general, resembled their parents and the cannabis produced therefrom was not usually distinguishable from that derived from first generation plants. Although cannabinoid patterns were, in some cases, similar to those of both parents and original seedstock cannabis, there were many exceptions. The yields of cannabis were significantly higher than from first generation plants, but the tetrahydrocannabinol contents were lower. Although higher than in the original imported cannabis, the tetrahydrocannabinolic acid to tetrahydrocannabinol ratios in cannabis from second generation plants were lower than in cannabis produced from first generation plants.

Introduction

It may be of considerable forensic importance to know whether a seizure of cannabis* in the United Kingdom was originally imported or produced from *Cannabis* illicitly cultivated in the United Kingdom. Baker, Gough and Taylor [1], therefore, studied the physical and chemical features of cannabis samples prepared from *Cannabis* plants grown in the United Kingdom from imported seeds of known geographical origin. It was concluded that the gross physical appearance and cannabinoid pattern of the samples were, in most cases, closely related to those of the parent (imported) samples. Wide variations in the actual tetrahydrocannabinol (THC) contents were noted between plants grown from seeds from different countries and within a given

* The term cannabis in this paper refers to marijuana, and the term *Cannabis* refers to *Cannabis sativa* L.

country. The cannabinoids were present mainly as their cannabinoid acids in these plants. The ratio of tetrahydrocannabinolic acid (THCA) to THC in the material grown in the United Kingdom was found, in general, to have been considerably higher than in imported samples of cannabis.

In view of the widespread illicit cultivation of *Cannabis* within the United Kingdom [2] and in consequence the likely availability of fertile seeds resulting from such cultivation, it was also necessary to study the physical and chemical features of *Cannabis* plants grown from seeds produced within the United Kingdom.

Fairbairn and Liebmann [3], in suggesting that there were two chemical races of *Cannabis* plants, one being THC-rich, the other being rich in cannabidiol (CBD), considered that this was under genetic control and independent of environmental conditions. These authors proposed that several generations of plants should be grown in order to study this hypothesis. In subsequent studies, Fairbairn and Rowan [4] and Rowan and Fairbairn [5], presented further evidence and found that THC- or CBD-rich patterns were established even when plants were small.

They also found that cannabichromene (CBCh) was absent in CBD-rich plants, although present in THC-rich plants. Boucher, Paris and Cosson [6] grew plants containing considerable amounts of tetrahydrocannabivarinic acid (THVA) and observed that within three generations the plants had become THCA-rich, a result which might indicate that THCA : THVA ratios might be under environmental control. Yotoriyama and others [7] grew *Cannabis* plants in Japan. When a cannabidiolic acid (CBDA)-rich plant was crossed with a THCA-rich plant, median offspring were produced which contained CBDA, THCA and cannabichromenic acid (CBChA). Offspring from this median variety fell into three groups: 29 per cent of the plants contained high levels of CBDA, 20 per cent contained high THCA levels and the remainder were as the median variety. Two subsequent generations bred from the high CBDA variety were both wholly high CBDA variety. Several further studies of licitly cultivated plants have been discussed in the authors' previous publication [1].

In order to study further cannabis produced within the United Kingdom, the authors felt it was necessary to grow *Cannabis* plants from seed produced in the United Kingdom. These seeds were taken from *Cannabis* plants of known cannabinoid content and parentage [1].

Experimental

Seed selection

As in the previous study [1], plants were separated according to sex as soon as flowering commenced. Female plants were carefully pollinated by hand in order to ensure that both parents of any fertile seeds were from the

same original seedstock and were of known cannabinoid content. Apart from the Indian plants, all subjects of this study were second generation, i. e. their parent plants were grown in the United Kingdom in 1980 from imported seeds of known origin. The Indian plants were third generation, the first generation having been grown in the United Kingdom in 1979 and the second in 1980.

Cultivation and harvesting

As far as possible, cultivation was carried out under identical conditions to those prevailing in 1980. Weather conditions, although obviously outside experimental control, were similar in the main growing seasons (June, July and August). In 1980 there were 514.3 hours of sunshine and a mean temperature of 16.9 °C. In 1981 the values were 515.6 hours of sunshine and a mean temperature of 17.2 °C and, in both years, June and July were colder than the long-term average, while August was warmer [8]. Plants were harvested at maturity except for those showing signs of early distress and the dates of harvesting are listed in table 1. By contrast to 1980, when maturation dates fell into two periods, July to August and November, the plants grown in 1981 matured from July through November. As in 1980, male Moroccan plants were earliest to mature, all having being harvested by mid-July and, within countries of origin, male plants were, in general, earliest to mature. The plants whose original seedstock was southern African (Zambia and Zimbabwe) were, as in 1980, latest to mature, one plant from each of these two countries growing into late November. In physical appearance, plants resembled their parents, particularly in shape, size and stem hue.

Analysis

After harvesting, the leaves and flowering parts of the plants were separated from the woody stems and stalks, air dried, and any remaining large stalks removed prior to weighing the resultant product from each plant. The yield by weight from each plant was higher in 1981 than in 1980, perhaps reflecting acclimatization by the plants to the United Kingdom environment (see table 1). Female plants, in general, yielded more cannabis than male plants from within a given country. Several plants produced over 100 g of cannabis each, amply demonstrating that large quantities of cannabis can be obtained from plants grown in a temperate climate. All the samples were analysed by thin-layer chromatography (TLC) [9], gas-liquid chromatography (GLC) [10] and high performance liquid chromatography (HPLC) [11]. The THCA content was determined by GLC and HPLC, as previously described [12], and THVA content was calculated by reference to the sample HPLC chromatogram using a previously estimated response factor relative to THC [11].

Table 1

Quantitative data on *Cannabis* plants grown in 1981

Country of origin and plant number[a]	*Harvesting date*	*Sex*[b]	*Weight of cannabis (g)*	*Content (percentage by weight)*					
				CBD[cd]	*THC*[c]	*THV*[ce]	*THCA*	*THC*	*THCA/THC*
India									
4.1	September	F	48	1.6	0.85	—	0.78	0.17	5
4.2	August	MF	106	1.4	0.61	—	0.64	0.05	13
4.3	August	MF	41	ND[d]	2.50	—	2.70	0.10	27
4.4	September	F	85	ND	2.80	—	3.00	0.13	23
5.1	September	F	72	1.0	0.58	—	0.23	0.38	0.6
5.2	August	MF	71	1.1	0.64	—	0.66	0.06	11
5.3	August	MF	32	1.3	0.73	—	0.76	0.06	13
5.4	September	F	44	1.1	0.71	—	0.76	0.04	19
Morocco									
1.1	September	F	87	0.3	0.40	—	0.36	0.08	4
1.2	August	F	83	ND	1.40	—	1.20	0.36	3
1.3	July	M	16	ND	0.53	—	0.32	0.25	1
3.1	July	M	38	ND	0.48	—	0.22	0.29	1
3.2	July	M	32	ND	0.75	—	0.64	0.19	3
3.3	July	M	44	ND	0.45	—	0.31	0.21	1

Sri Lanka									
1.1	August	F	100	1.9	1.30	—	1.30	0.14	9
1.2	August	M	86	1.6	1.10	—	1.20	0.09	13
1.3	September	F	189	1.2	1.30	—	1.40	0.11	8
3.1	August	F	96	1.3	0.77	—	0.81	0.06	13
3.2	July	M	35	2.8	0.13	—	0.10	0.04	2
3.3	August	F	118	2.1	0.11	—	0.10	0.02	5
Zambia									
1.1	October	M	32	ND	0.43	2.20	0.38	<0.1	>4
1.2	October	F	50	ND	0.66	0.98	0.64	<0.1	>6
1.3	September	M	24	ND	2.00	2.10	2.20	<0.1	>22
3.1	November	F	71	ND	0.29	1.40	0.22	<0.1	>2
3.2	September	I	57	ND	0.70	4.60	0.68	<0.1	>7
3.3	October	F	43	ND	0.29	2.10	0.22	<0.1	>2
Zimbabwe									
2.1	September	I	20	ND	1.40	1.80	1.50	<0.1	>15
2.2	November	F	41	ND	1.70	0.66	1.80	<0.1	>18
2.3	October	F	31	ND	0.96	1.30	1.00	<0.1	>10
2.4	October	M	45	ND	0.88	1.50	0.88	<0.1	>9

[a] The first digit identifies the female parent plant grown in 1980 (see table 2, reference [1]).
[b] F = female plant, M = male plant, I = immature plant.
[c] As determined by GLC i. e. includes decarboxylated THCA.
[d] ND = not detected.
[e] In the samples from India, Morocco and Sri Lanka the THV content was very much smaller than the THC content.

Results and discussion

All of the cannabis prepared from plants harvested in 1981 was yellow or yellow-green friable material which was not visually distinguishable from the 1980 products and, in some cases, not distinguishable from the original imported cannabis.

In many cases, cannabinoid patterns, as determined by TLC, were similar to that of the original imported cannabis from which the seedstock was taken. Plants from southern African seedstock (Zambia and Zimbabwe) produced cannabis with cannabinoid patterns similar in both first and second generation crops: levels of THV were, in all cases (10 samples), comparable with or greater than those of THC. Unlike Boucher, Paris and Cosson [6], the authors did not find significantly different THV : THC ratios in plants of different generations. The original Moroccan seedstock cannabis (i.e. the imported material), contained CBD. Only one of the three plants grown in 1980 and one of six plants grown in 1981, however, contained CBD. The Sri Lankan seedstock cannabis contained CBD, as did two of the three plants grown in 1980. All six plants grown in 1981 contained CBD, as did seven seedlings analysed by TLC only after four weeks of growth (i.e. considerably before maturity).

In the light of the results of Fairbairn and Rowan [4], and Rowan and Fairbairn [5], it seems likely that these would have been CBD-containing plants at maturity. Neither seedstock cannabis, nor first generation Indian plants (grown in 1979) contained CBD, but one of the five second generation plants (grown in 1980) and six of the eight third generation plants (1981) contained CBD. HPLC and TLC indicated the presence of cannabichromene (CBCh) in two (Sri Lanka, No. 3.2 and India, No. 5.2), of the 14 CBD-containing plants grown in 1981. Rowan and Fairbairn [5] found no CBCh in CBD-rich seedlings. The results are summarized in figures I to V

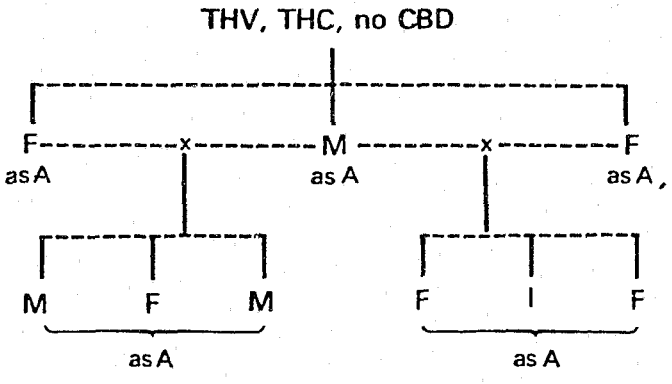

Figure I
Seedstock cannabis (Zambia)

which show the cannabinoids by TLC on plants grown in the United Kingdom. The key is as follows:

I: Immature plants which died before flowering.
M: Male plant.
F: Female plant.
MF: Male/female plant.

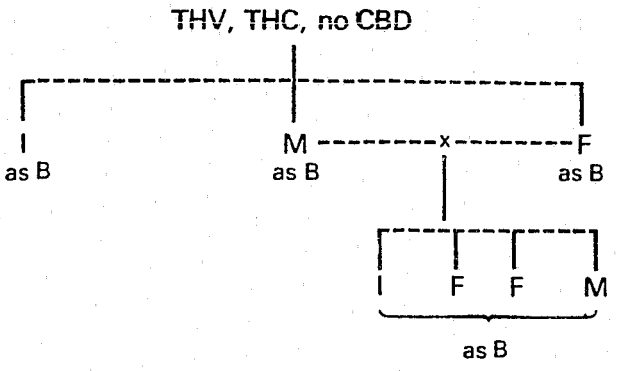

Figure II
Seedstock cannabis (Zimbabwe)

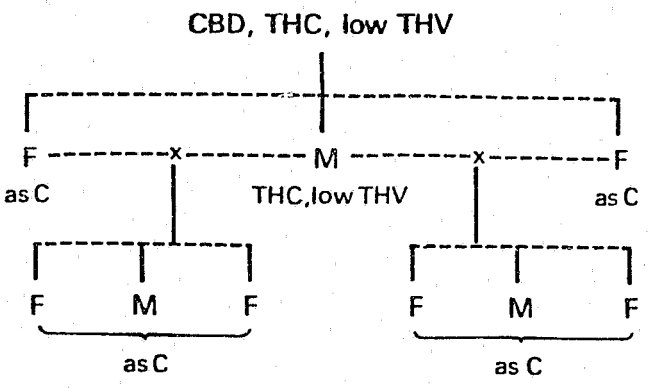

Figure III
Seedstock cannabis (Sri Lanka)

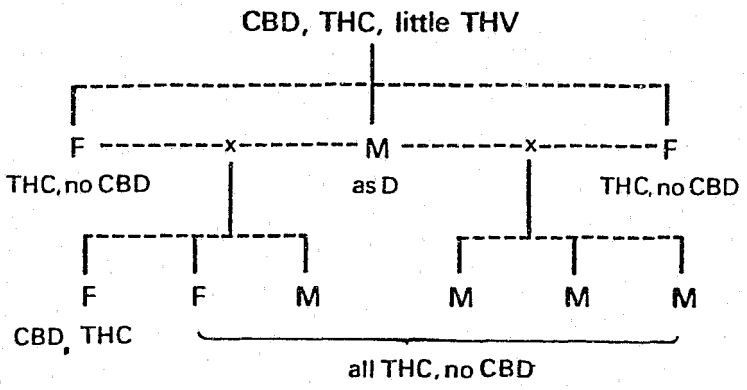

Figure IV
Seedstock cannabis (Morocco)

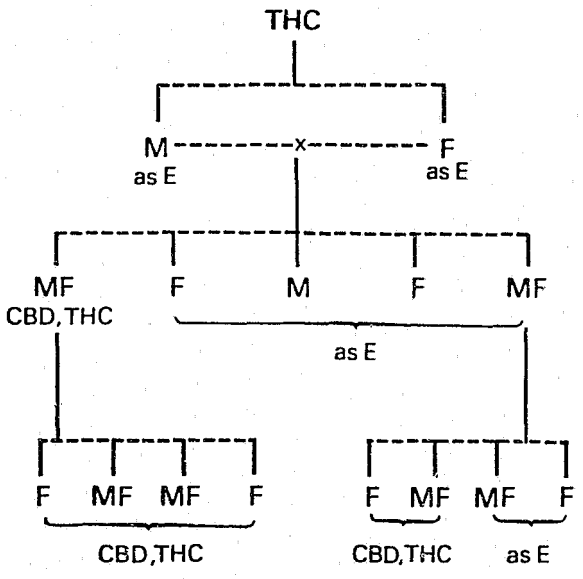

Figure V
Seedstock cannabis (India)

Quantitative data

The thermally labile cannabinoid acids are decarboxylated during gas chromatographic analysis to the corresponding cannabinoids [13, 14]. Quantitative measurements of the amount of a given cannabinoid by GLC therefore gives the total sum of the cannabinoid and its acid.

Table 2

CBD/THC ratios in seedstock cannabis and *Cannabis* grown in the United Kingdom

Plant origin		*Generation*	*CBD/THC*
Morocco		Imported sample	0.57
Sri Lanka		Imported sample	1.1
India	5	2nd (1980)	2.5
Morocco	2	1st	1.6
Sri Lanka	1	1st	20
Sri Lanka	3	1st	17
India	4.1	3rd (1981)	1.9
India	4.2	3rd	2.3
India	5.1	3rd	1.7
India	5.2	3rd	1.7
India	5.3	3rd	1.8
India	5.4	3rd	1.5
Morocco	1.1	2nd	0.75
Sri Lanka	1.1	2nd	1.5
Sri Lanka	1.2	2nd	1.6
Sri Lanka	1.3	2nd	0.92
Sri Lanka	3.1	2nd	1.7
Sri Lanka	3.2	2nd	22
Sri Lanka	3.3	2nd	19

The original imported cannabis samples and cannabis produced from plants grown in 1980 and 1981 could be divided into three categories on the basis of CBD/THC ratios as determined by GLC; i. e. those where the ratio exceeded 10, those where the ratio was approximately unity and those where the ratio was zero (i. e. the sample contained no detectable CBD). These results are summarized in table 2. It is possible that the plants with a large amount of CBDA obtained by Yotoriyama and others [7] in their study might correspond to the plants grown in the United Kingdom with CBD:THC ratios greater than 10. It is also likely that the median variety corresponds to plants with a CBD:THC ratio of approximately unity. No plants in the authors' studies corresponded to those having a high THCA content and a low CBDA content. The results of analysis of second generation plants grown from Sri Lankan seeds where two high CBD plants were crossed with a CBD-absent plant indicate that four median varieties and two high CBD varieties were produced. This result indicates that there may be considerable variation in the plant population of that country. Small and Beckstead [15] and Small, Beckstead and Chan [16] also considered that variations might exist within populations of *Cannabis*. All the Moroccan plants grown in 1980 and 1981 were either CBD-free or of the median variety. There was no evidence for high-CBD plants either in seizures of Moroccan cannabis examined at the Laboratory of the Government Chemist or in any of the plants grown in the United Kingdom. It may well be

that the high CBD plants which are generally considered to be those from which hemp fibre is produced have been removed from the population, either by selective breeding or by harvesting before maturity in order to produce a *Cannabis* crop suitable for illicit drug preparation.

The appearance of median CBD-containing plants in second and third generation Indian plants may also indicate considerable plant population variation in that country. Many seizures of cannabis from India do contain CBD [1] and inter-breeding may occur. It should, however, not be overlooked that environment may also influence whether or not a plant produces CBD. There was no evidence for the presence of CBD in any plants grown from southern African seedstock either in 1980 or 1981, and cannabinoid patterns were similar. It would seem that these were stable populations.

Actual THC contents, as determined by GLC, were lower in 1981 (mean THC content 0.9 per cent) as compared with 1980 (mean THC content 2.2 per cent). Much higher yields of cannabis (by weight) were, however, obtained in 1981 (mean yield 61 g per plant), compared with 1980 (mean yield 30 g per plant). The mean weights of THC per plant were similar in the two years, being 0.66 g and 0.57 g for 1980 and 1981 respectively. Thus, although much lower yields of cannabis were obtained in 1980, this was compensated for by higher levels of THC.

Most of the cannabinoids were present in the harvested material as cannabinoid acids. THCA : THC ratios in the harvested plants were much lower in 1981 (mean 9.4, $s=7.4$) than in 1980 (mean 40, $s=46$), but still considerably higher than the value of this ratio for all samples ($n=64$) of fresh cannabis imported illicity into the United Kingdom in 1979 (mean 2.4, $s=2.2$). The high levels of THVA in plants from southern Africa made accurate measurement of THC contents difficult as the two cannabinoids were not adequately resolved by HPLC when a considerable excess of THVA was present in the cannabis extract. Full quantitative data are summarized in table 1.

Conclusions

Cannabis, produced from imported seedstock of known geographical origin, grown over at least two generations bears, in many cases, considerable physical resemblance to the original imported cannabis. With some exceptions, there were considerable variations in cannabinoid patterns of plants as determined by TLC, even when plants were grown from the same seedstock, possibly indicating considerable variations in the plant populations in the country from which the seedstock originated. Mean yields of cannabis were considerably higher in 1981 when compared with 1980, but THC contents were much lower. The mean THCA : THC ratios in 1981 were

much lower than in 1980, but still appreciably higher than in fresh imported cannabis. Further generation studies are being undertaken to monitor any further variations in plant characteristics.

References

1. P. B. Baker, T. A. Gough and B. J. Taylor, "The physical and chemical features of *Cannabis* plants grown in the United Kingdom of Great Britain and Northern Irelands from seeds of known origin", *Bulletin on Narcotics* (United Nations publication), vol. 34, No. 1 (1982), pp. 27 – 36.
2. B. Connett, Metropolitan Police Forensic Science Laboratory, Private Communication (London).
3. J. W. Fairbairn and J. A. Liebmann, "The cannabinoid content of *Cannabis sativa* L. grown in England", *Journal of Pharmacy and Pharmacology,* vol. 26, 1974, pp. 413 – 419.
4. J. W. Fairbairn and M. G. Rowan, "Cannabinoid patterns in *Cannabis sativa* L. as an indication of chemical race", *Journal of Pharmacy and Pharmacology,* vol. 27, 1975, p. 90P.
5. M. G. Rowan and J. W. Fairbairn, "Cannabinoid patterns in seedlings of *Cannabis sativa* L. and their use in the determination of chemical race", *Journal of Pharmacy and Pharmacology,* vol. 29, 1977, pp. 491 – 494.
6. F. Boucher, M. Paris and L. Cosson, "Mise en évidence de deux types chimiques chez le *Cannabis sativa* originaire d'Afrique du Sud", *Phytochemistry,* vol. 16, 1977, pp. 1445 – 1448.
7. M. Yotoriyama and others, "Plant breeding of cannabis. Determination of cannabinoids by high-pressure liquid chromatography", *Yakugaku Zasshi,* vol. 100, 1980, pp. 611 – 614.
8. The Meteorological Office, Bracknell, Berkshire, United Kingdom of Great Britain and Northern Ireland.
9. R. Fowler, R. A. Gilhooley and P. B. Baker, "Thin-layer chromatography of cannabinoids", *Journal of Chromatography,* vol. 171, 1979, pp. 509 – 511.
10. P. B. Baker and others, "Variation in the THC content in illicitly imported *Cannabis* products", *Bulletin on Narcotics* (United Nations Publication), vol. 32, No. 4 (1980), pp. 47 – 54.
11. P. B. Baker and others, "Determination of the distribution of cannabinoids in cannabis resin using high performance liquid chromatography", *Journal of Analytical Toxicology,* vol. 4, 1980, pp. 145 – 152.
12. P. B. Baker, B. J. Taylor and T. A. Gough, "The tetrahydrocannabinol and tetrahydrocannabinolic acid content of *Cannabis* products", *Journal of Pharmacy and Pharmacology,* vol. 33, 1981, pp. 369 – 372.
13. A. Ohlsson and others, "Cannabinoid constituents of male and female *Cannabis sativa*", *Bulletin on Narcotics* (United Nations Publication), vol. 23, No. 1 (1971), pp. 29 – 32.

14. P. S. Fetterman and others, "A simple gas liquid chromatography procedure for determination of cannabinoidic acids in *Cannabis sativa* L.", *Experientia*, vol. 27, 1971, pp. 988 – 990.

15. E. Small and H. D. Beckstead, "Cannabinoid phenotypes in *Cannabis sativa*", *Nature*, vol. 245, 1973, pp. 147 – 148.

16. E. Small, H. D. Beckstead and A. Chan, "The evolution of cannabinoid phenotypes in *Cannabis*", *Economic Botany*, vol. 29, 1975, pp. 219 – 232.