Constituents of *Cannabis sativa* L. II: Absence of Cannabidiol in an African Variant

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Abstract □ Cannabidiol is shown to be absent in an African variant of *Cannabis sativa* L. (marijuana) grown in Mississippi. TLC, GC, and GC–mass spectrometry were used for identification. The absence of cannabidiol in a variant of African Cannabis questions the validity of published biosyntheses of the cannabinoids.

Keyphrases □ *Cannabis sativa* L., African variant grown in Mississippi—absence of cannabidiol, cannabinoid biosynthesis questioned □ Cannabidiol absence in African *C. sativa* grown in Mississippi—role in cannabinoid biosynthesis questioned □ Biosynthesis of cannabinoids—questioned due to absence of cannabidiol in African *C. sativa* grown in Mississippi

De Faubert Maunder (1) reported that cannabidiol is definitely absent in some samples of Cannabis and put forth the hypothesis that Cannabis plants grown south of the Sahara may have a different biogenesis from plants grown in the Middle East. Moreover, Korte *et al.* (2) reported an absence of cannabidiol in extracts of hashish from Cyprus, Nigeria, and Brazil. These hashish samples were obtained from plants grown using seed obtained from the United Nations and coded as UNC-33, 59, and 61, respectively. Additionally, Schou and Nielsen (3) reported zero for the extinction maximum (nm.) for the azo-dye derivative of cannabidiol from extracts of Indian, Brazilian, and UNC-135 Cannabis. Schou and Nielsen also reported that South African Cannabis from UNC-134 seed stock grown in Denmark contained only traces of cannabidiol regardless of the plant part analyzed. Although the aforementioned Cannabis preparations were analyzed primarily by TLC, Vree *et al.* (4) used combined GC–mass spectrometry to ascertain that a Brazilian sample of Cannabis contained no cannabidiol.

Samples analyzed by De Faubert Maunder (1), Korte *et al.* (2), Schou and Nielsen (3), and Vree *et al.* (4) contained measurable amounts of (−)-Δ^9-*trans*-tetrahydrocannabinol and cannabinol. Korte *et al.* also reported that UNC-33, 59, and 61 contained measurable amounts of tetrahydrocannabinol II, which has since been identified as cannabichromene (5, 6). Vree *et al.* noted that the propyl derivative of cannabinol and cannabichromene could not be easily separated by GC but that the propyl derivative of cannabinol could be easily identified because of its characteristic mass fragments at 282 and 267. Although one published report (6) gives slightly different retention times for cannabidiol and cannabichromene, this laboratory and others (7) have been unsuccessful in obtaining a neat separation of cannabidiol and cannabichromene using only GC. Therefore, three cannabinoids (cannabichromene, cannabidiol, and cannabivarin[1]) are practically impossible to identify using relative retention times only. This fact, as well as the apparent absence of cannabidiol but presence of cannabichromene in certain samples of Cannabis, prompted an investigation of this problem using TLC, GC, and combined GC–mass spectrometry.

Additionally, it seemed worthwhile to examine De Faubert Maunder's (1) proposal that a "different biogenesis" than that originally proposed by Todd (8) and Simonsen and Todd (9) and modified by Mechoulam (10) and Mechoulam and Gaoni (11) may be operative in certain "sub-species of cannabis." Although Schou and Nielsen (3) did not suggest that a different biogenesis may be operative in certain variants of Cannabis, they did state that "the insignificant content of cannabidiol seems to be a characteristic feature" of UNC-134 from South Africa. To ascertain if, indeed, cannabidiol is absent in a particular variant of Cannabis, several variants grown in Mississippi from known origin were investigated.

METHODS

The samples used were grown in Mississippi from seed obtained through the National Institute of Mental Health (NIMH), the U. S. Department of Agriculture (USDA), and the United Nations (UN). The plant material was grown in Mississippi in 1970, 1971, and 1972. Samples were taken of various aged plant material. Each sample, containing aboveground plant parts, was taken from four different plants and dried at 22 ± 2° for 4 days. They were manicured by passing the material through a 14-mesh sieve; this process removed all large stems and fruits.

GC Analyses—The extraction procedure was basically that described by Lerner (12) and Fetterman *et al.* (13) with minor modifications. Three 1-g. samples were extracted simultaneously with 40 ml. of spectrograde chloroform. The resulting solutions were refrigerated at 6° and shaken at 10-min. intervals for 1 hr.; the plant material then was removed by filtration, and the mother liquor was concentrated *in vacuo* at ambient temperature to a greenish paste void of solvent. This concentrate was then dissolved in approximately 1 ml. of ethanol. The ethanolic solution was subjected to continuous vibration from an ultrasonic vibrator until all resin was in solution. At this point, an additional 0.5 ml. of an ethanolic solution of 4-androstene-3,17-dione was added as the internal standard. The concentration of the internal standard required will vary depending upon the age of the plant material. Usually from 0.05 to 0.5 μl. of this solution was injected into the chromatograph.

Analyses were performed using gas chromatographs[2] equipped with hydrogen flame-ionization detectors and operated isothermally at 210°. The inlet temperature and detector temperature were 240 and 260°, respectively. Glass columns, 0.63 cm. (0.25 in.) o.d. and 2 mm. i.d. × 2.43 m. (8 ft.), were packed with 2% OV-17 (high purity polar phenyl methyl silicone; approximately 30,000 mol. wt.) on 100–120-mesh Chromosorb "WHP." Nitrogen was used as the carrier gas at a flow rate of 10–30 ml./min., depending upon separation requirements, *etc.* Peak area measurements were made using the method of peak height times width at half height. The peak area of each cannabinoid was compared with the peak area of the internal standard, and the cannabinoid concentration was

[1] *n*-Propyl homolog of cannabinol. (See *References 15, 22,* and *23.*)

[2] Beckman GC-45 and GC-72-5.

Table I—Analyses[a] of South African (A-A) *C. sativa* L.

Generation	Sex	Age, weeks	V and XI	VII	XIII	VI	VIII
First	Mixture[b]	16	0.17	0.11	Trace	1.60	0.03
Second	Mixture	16	0.05	0.27	0.02	1.18	0.03
Third	Young[c]	9	0.12	0.06	0.15	0.84	0.05

[a] Key: V, tetrahydrocannabivarin; XI, cannabicyclol; VII, cannabichromene; XIII, cannabigerol monomethyl ether; VI, Δ^9-tetrahydrocannabinol; and VIII, cannabigerol. [b] Contains mature male and female plants. [c] Plant material analyzed prior to sexual differentiation.

determined by using the appropriate relative response factor. Synthetic cannabinoids used in determining the relative response factors were obtained through NIMH.

TLC (Qualitative)—Extracts for TLC were prepared as described for GC with the exception of adding the internal standard. Plates were precoated with 0.25 mm. of silica gel G[3] without fluorescent indicator. Plates were then impregnated according to the procedure described by Korte and Sieper (14), using dimethylformamide in carbon tetrachloride at a ratio of 6:4. Development was in saturated chambers to a height of 15 cm. above the starting point. The temperature of the room used for thin-layer work was maintained at 20 ± 1°. Relative humidity was maintained at 50–60% by a dehumidifier[4]. The solvent systems were: (1) cyclohexane (14), using the procedure described by Merkus (15); (2) hexane–toluene (3:2); (3) hexane–xylene (3:1); (4) hexane–xylene–acetone at various ratios; (5) ethyl acetate–hexane (1:1) (16); and (6) petroleum ether–ether (4:1) as described by Machata (17). Fast Blue Salt B[5] was the spray reagent (0.5% aqueous solution).

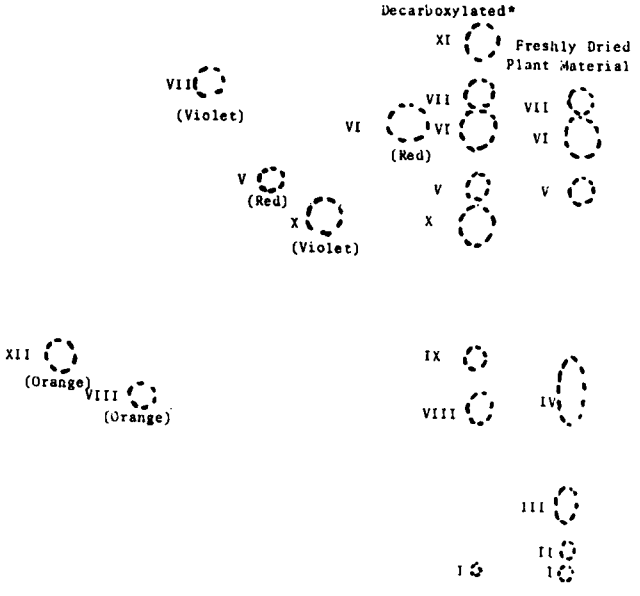

Figure 1—*Thin-layer chromatogram of South African (A-A) C. sativa L. plate was impregnated with dimethylformamide and developed twice with petroleum ether–ether (4:1). Spray reagent was Fast Blue Salt B, 0.5% aqueous solution. Key: I, origin; II, cannabigerolic acid (orange); III, (−)-Δ^9-trans-tetrahydrocannabivarinic acid (red); IV, (−)-Δ^9-trans-tetrahydrocannabinolic acid (red); V, (−)-Δ^9-trans-tetrahydrocannabivarin (red); VI, (−)-Δ^9-trans-tetrahydrocannabinol (red); VII, cannabichromene (violet); VIII, cannabigerol (orange); IX, cannabivarin (violet); X, cannabinol (violet); XI, cannabicyclol (red); and XII, cannabidiol (orange). (* Material was prepared by heating the plant material at 100° for 1 hr.)*

Table II—Relative Retention Times of Cannabinoids[a]

	Relative Retention Time[b]
Olivetol	0.04
Cannabidivarin	0.18
Tetrahydrocannabivarin	0.26
Cannabicyclol	0.26
Cannabichromene	0.34
Cannabivarin	0.34
Cannabidiol	0.34
Cannabigerol monomethyl ether	0.38
$\Delta^{9,11}$-Tetrahydrocannabinol (exocyclic)	0.41
Δ^8-Tetrahydrocannabinol	0.44
Δ^9-Tetrahydrocannabinol	0.49
Cannabigerol	0.57
Cannabinol	0.63
4-Androstene-3,17-dione	1.00
Unknown	1.18
Unknown	1.29
Unknown	1.38

[a] 4-Androstene-3,17-dione was used as the internal standard. [b] Relative retention times were calculated using synthetic cannabinoids and Cannabis plant material. Column conditions were described previously under *Methods* section.

GC–Mass Spectrometry—A gas chromatograph[6], equipped with a hydrogen flame-ionization detector, in combination with a high-resolution mass spectrometer[7], equipped with a jet separator for sample enrichment, was utilized. A stainless steel column, 0.31 cm. (0.125 in.) o.d. × 1.52 m. (5 ft.) packed with 2% OV-17 on Supelcoport was used as previously described. Helium was the carrier gas, and the mass spectrometer was operated at 70 ev.

RESULTS AND DISCUSSION

Cannabichromene, referred to as tetrahydrocannabinol II by Korte *et al.* (2), has been isolated and characterized by several researchers (5, 18–20). The biological activity has also been studied. Gaoni and Mechoulam (19) originally reported that cannabichromene was active in the dog assay. Later Isbell *et al.* (20) reported that they observed no activity in humans. Mechoulam *et al.* (21) concluded that, except for (−)-Δ^9-*trans*-tetrahydrocannabinol, there was no active constituent in a sample of hashish analyzed to contain 0.1% cannabichromene. To minimize conflicts in this report, only variant(s) of Cannabis from known geographical origin were investigated. In each case the three investigative methods previously described were employed.

Since most previous reports pertaining to the absence of cannabidiol and/or the presence of cannabichromene (1–4) were from parts of Africa or Brazil, it was decided that a variant of Cannabis from South Africa would best serve as a vehicle for: (*a*) ascertaining if there is a total absence of cannabidiol in a certain variant of Cannabis and (*b*) exploring the possibility of a potential "different biogenesis" for certain variants of Cannabis.

Three successive generations of South African Cannabis (UNC-255), coded as A-A, were grown in Mississippi in 1970, 1971, and 1972. Samples of the young material were taken in 1970 and 1971 prior to and after sexual maturity; in 1972, samples were taken each week. Each sample was prepared for analysis as previously described.

TLC was carried out routinely, as was GC, on fresh plant material from the 1972 plantings. The thin-layer systems previously mentioned were utilized; however, the system of petroleum ether–ether at a ratio of 4:1 gave best results in these laboratories. Plates impregnated with dimethylformamide in carbon tetrachloride at a ratio of 6:4, as described by Korte and Sieper (14), were removed from the storage tank and dried approximately 1 min. with an air blower[8] set on cool. The plate was spotted 1.5 cm. from the bottom and was then placed in the developing tank. Usually the time period from removal of the plate to immersion in the developing tank was

[3] Merck.
[4] Sears Coldspot.
[5] Merck.
[6] Varian series 1400.
[7] Du Pont 21-492.
[8] Heat-Blo.

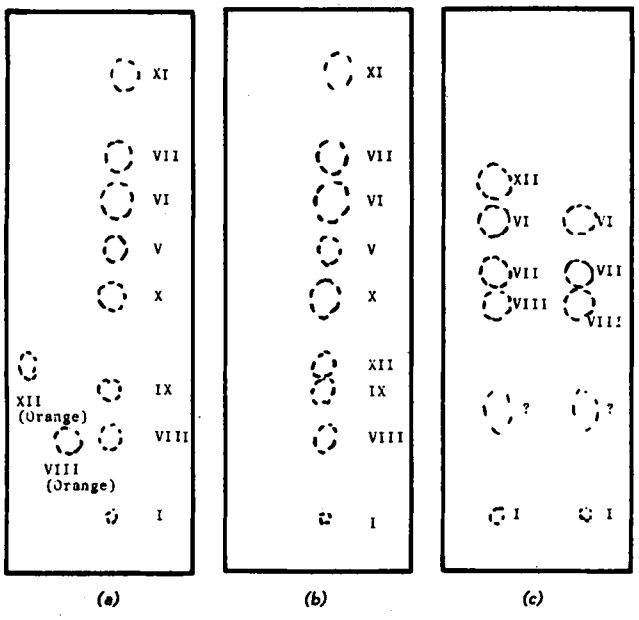

Figure 2—(a) *Thin-layer chromatogram of A-A decarboxylated plant material. Plate was impregnated with* N,N-*dimethylformamide. Solvent system was the same as described for Fig. 1.* (b) *Thin-layer chromatogram of A-A decarboxylated with synthetic cannabidiol added. Plate was developed according to procedures described for Figs. 1 and 2a.* (c) *Thin-layer chromatogram of A-A decarboxylated plant material (right) and decarboxylated plant material with synthetic cannabidiol (left). Plate was developed as described for preceding figures. Plate was not impregnated with* N,N-*dimethylformamide.*

no more than 10 min. The plates were developed one time, air dried for 1 min., replaced in the chamber, and developed a second time with subsequent air drying for 1 min. The plate was then sprayed with a 0.5% solution of Fast Blue Salt B in water.

Merkus (15, 22, 23) used cyclohexane as the developing solvent and developed each plate three times to obtain excellent separation. However, in our hands the petroleum ether–ether system afforded comparable separation at approximately half the time. In addition, impregnated plates in these laboratories showed no evidence to indicate destruction of the impregnation as was previously observed (3).

Evidence was found to support the contention by Merkus (15) that hydrogen bonding between the stationary phase, dimethylformamide, and the phenolic group of the cannabinoids was stronger in the *n*-propyl homologs than the *n*-amyl. Moreover, when hexane–toluene in a ratio of 55:45 was used to separate the acids of (−)-Δ^9-*trans*-tetrahydrocannabinol and (−)-Δ^9-*trans*-tetrahydrocannabivarin[9], hydrogen bonding was more pronounced in the propyl homolog. Tentative evidence indicates that the *n*-propyl homologs undergo decarboxylation slower than the *n*-amyl; however, the *n*-propyl homologs tend to oxidize easier. More data will be forthcoming on this subject.

The thin-layer chromatogram of African Cannabis, using impregnated plates with petroleum ether–ether as described by Merkus (15) and De Zeeuw *et al.* (24, 25), shows a distinct, elongated spot for (−)-Δ^9-*trans*-tetrahydrocannabinolic acid and (−)-Δ^9-*trans*-tetrahydrocannabivarinic acid. Other acids of the cannabinoids are near the origin (Fig. 1). Additionally there are spots for the neutral decarboxylated cannabinoids. Cannabichromene is located near the solvent front followed by (−)-Δ^9-*trans*-tetrahydrocannabinol and (−)-Δ^9-*trans*-tetrahydrocannabivarin. No cannabidiol is observed. Additionally, when all cannabinoids are decarboxylated to their neutral derivatives (Figs. 1 and 2*a*), cannabidiol is still undetectable. However, when cannabidiol is added to the sample, a corresponding spot is observed (Fig. 2*b*).

When utilizing the same solvent system with unimpregnated plates, cannabidiol is near the solvent front (24, 25). In Fig. 2*c*, a sample enriched with cannabidiol is compared with a sample of regular plant material. Although thin-layer evidence strongly suggests that cannabidiol is absent in African Cannabis grown in Mississippi, the sample was then subjected to further analysis by GC.

GC analyses of the first, second, and third generations of African (A-A) Cannabis showed some fluctuation in the cannabinoid content (Table I). The possibility that hybridization caused some juxtaposition of the cannabinoids cannot be overlooked, since there were no major attempts to prevent cross-pollination of the growing area. However, similar variants were grown in the same area taking advantage of the prevailing winds. Certain variants were planted prior to others to minimize further cross-pollination. Figure 3, a chromatogram of the second generation, is typical of the African variant and contains a peak corresponding to cannabidiol. The dotted overlay represents the increase in intensity of the peak normally attributed to cannabidiol when spiked with synthetic cannabidiol. At first, this would seem to indicate that cannabidiol is, indeed, present in plant material from Africa; however, Table II of the relative retention times shows cannabidiol, cannabichromene, and cannabivarin are, for all practical purposes, inseparable under the conditions stated previously. Therefore, the sample was subjected to combined GC–mass spectrometry analysis.

The mass spectra data obtained from A-A plant material is given in Table III. Spectra of synthetic cannabidiol and cannabichromene were used as standards. The absence of a significant *m/e* at 286, 260, 259, 246, 229, and 230 from the plant material is indicative of an absence of cannabidiol. Cannabidiol has an *m/e* 246 of strong intensity, which was very hard to distinguish from background noise in A-A. Present in the plant material were *m/e* 267, 257, and 243; all were found in synthetic cannabichromene but were absent or

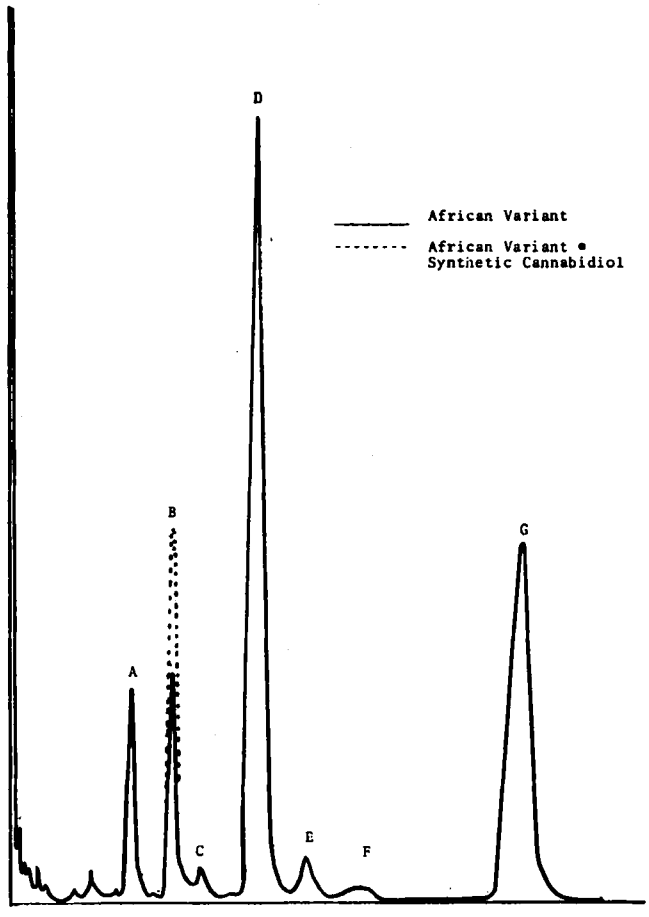

Figure 3—*Chromatogram of African* C. sativa *L. coded A-A (second generation). Key: A,* (−)-Δ^9-trans-*tetrahydrocannabivarin and/or cannabicyclol; B, cannabidiol, cannabichromene, cannabivarin, or any combination of these cannabinoids (in this variant, only cannabichromene is present); C, cannabigerol monomethyl ether; D,* (−)-Δ^9-trans-*tetrahydrocannabinol; E, cannabigerol; F, cannabinol; and G, 4-androstene-3,17-dione (internal standard).*

[9] *n*-Propyl homolog of (−)-Δ^9-*trans*-tetrahydrocannabinol.

Table III—Mass Spectra Data[a]

Compound[b]	m/e														
XII	314	299	286	271	—	260	259	258	—	246	—	232	231	230	229
VII	314	299	—	271	267	—	—	258	257	—	243	232	231	—	—
A-A	314	299	—	271	267	—	—	258	257		243	232	231	—	—

[a] Data obtained from combined GC–mass spectrometry as described under *Methods* section. [b] Key: XII, cannabidiol; and VII, cannabichromene.

insignificant in cannabidiol. The molecular ion for both cannabidiol and cannabichromene was at *m/e* 314 and the base peak was at *m/e* 231. These data correspond to published data for cannabidiol (26) and cannabichromene (5). Additional mass spectra data on the cannabinoids can be found in *References 27–31*.

Thus, TLC and GC–mass spectrometry indicate that each generation of the African variant (A-A) grown in Mississippi does not contain cannabidiol but rather contains cannabichromene, which can easily be erroneously reported as cannabidiol using only conventional GC methods. Moreover, from past experiences, TLC methods, even with pure reference standards, cannot be used independently to identify cannabinoids positively. For positive identification, it is best to use a combination of the three investigative tools described.

In continuation of the investigation of the cannabinoids in A-A, several interesting observations were made. Table I contains the percent of cannabinoids analyzed from A-A using GC after the constituents were identified by GC–mass spectrometry. As previously mentioned, the cannabinoid content does fluctuate from the first through the third generation when grown in Mississippi.

Analyses reported for the first and second generations of A-A were at 16 weeks, and the one for the third generation was at 9 weeks. Published (32) and unpublished reports from these laboratories showed that the cannabinoid content of different variants of Cannabis varies with age; thus, the fluctuation of major cannabinoids can be explained as a factor of age and/or possible hybridization. However, the increase in cannabigerol monomethyl ether from the first through the third generation cannot be adequately explained. Mass spectral data of the peak in Fig. 3 labeled cannabigerol monomethyl ether do match the data published by Yamauchi *et al.* (33). The molecular ion at *m/e* 330 corresponds with the fragmentation described by Yamauchi *et al.*

Additionally, the peak (Fig. 3) labeled (−)-Δ^9-*trans*-tetrahydrocannabivarin and cannabicyclol in this variant (A-A) was a mixture. Cannabicyclol is formed from cannabichromene (34) and could possibly be the product of cyclization in the injector port of the gas chromatograph. Moreover, cannabicyclol is formed when a fresh sample of Cannabis is heated (Fig. 1). Tentative data seem to indicate that samples containing cannabichromene and cannabidiol can be qualitatively analyzed by cyclization of the cannabichromene to cannabicyclol while cannabidiol, in these laboratories, remains unchanged.

(−)-Δ^9-*trans*-Tetrahydrocannabivarin was originally reported by Gill *et al.* (35) to be a constituent in the tincture of Cannabis. Later, Fetterman and Turner (30) reported its presence in a variant of Indian origin grown in Mississippi. (−)-Δ^9-*trans*-Tetrahydrocannabivarin has been observed in several variants grown in Mississippi and is probably present in most *C. sativa* L. samples in varying amounts. Additionally, as with most cannabinoids, the *n*-propyl homologs exist in fresh plant material as their carboxylic acid derivatives. Decarboxylation of fresh plant material containing (−)-Δ^9-*trans*-tetrahydrocannabivarinic acid, by heating at 100° for 1 hr., produced (−)-Δ^9-*trans*-tetrahydrocannabivarin and cannabivarin.

According to the published biogenesis of Cannabis (8–11), it is very improbable that cannabidiol could be absent in any plant sample; moreover, by using the three investigative tools described herein, it is highly unlikely that cannabidiol could be present and remain undetected. Thus, until adequate biogenesis experiments using tagged materials are reported, it is the contention of these authors that three possibilities exist:

1. A different biogenesis is operative in the African (A-A) and possibly other variants of Cannabis.
2. An error exists in the accepted structure of cannabichromene.
3. An enzymatic system is operative in certain variants that facilitates a concomitant allylic rearrangement of hydroxycannabigerol and cyclization of the rearranged intermediate to (−)-Δ^9-*trans*-tetrahydrocannabinol, bypassing or preventing the detection of cannabidiol. Numbers one and three are the most logical, whereas number two appears to be very remote.

SUMMARY

Cannabidiol is absent in a South African (A-A) variant of *C. sativa* L. obtained from seed stock UNC-225 furnished by the UN and grown in Mississippi. Three investigative tools were employed in a concerted effort to obtain physical data on the presence of cannabidiol without any positive results. Cannabichromene was found to be present in A-A and was cyclized to cannabicyclol by heating at 100° for 1 hr. When analyzed only by GC, cannabichromene, cannabidiol, and cannabivarin can easily be erroneously reported since they have the same relative retention time.

The absence of cannabidiol in a variant of Cannabis indicates the need for biogenesis experiments using tagged intermediates. The biogenesis currently accepted is not adequate without cannabidiol serving as an intermediate in the synthesis of (−)-Δ^9-*trans*-tetrahydrocannabinol.

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Production of Hypertension with Desoxycorticosterone Acetate-Impregnated Silicone Rubber Implants

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Abstract □ A simple method for the production of drug-impregnated silicone rubber implants for sustained-release drug administration is described. This method involves incorporating the drug in unpolymerized silicone rubber, adding catalyst, and casting the drug–silicone rubber mixture in a hemicylindrical shape in a methacrylate mold. The utility of this method was investigated with desoxycorticosterone acetate. Desoxycorticosterone acetate-impregnated silicone rubber implants were inserted subcutaneously in rats, unilateral nephrectomy was performed, and the rats were maintained on 1% sodium chloride solution in place of drinking water. Systolic blood pressure was determined by an indirect tail cuff method. Rats receiving doses of desoxycorticosterone acetate, ranging from 50 to 500 mg./kg., developed hypertension within 3 weeks. Desoxycorticosterone acetate in a dose of 100 mg./kg. appeared to be most suitable for inducing sustained hypertension. The advantages of this method for the production of experimental hypertension and the general applicability of this method for sustained-release drug administration are discussed.

Keyphrases □ Desoxycorticosterone acetate-impregnated silicone rubber implants—preparation, used to induce hypertension, rats □ Silicone rubber implants, desoxycorticosterone acetate impregnated—preparation, used to induce hypertension, rats □ Hypertension—induced by desoxycorticosterone-impregnated silicone rubber implants, rats □ Implants, desoxycorticosterone acetate-impregnated silicone rubber—preparation, used to induce hypertension, rats

Dimethylpolysiloxane has been shown to be inert in the presence of many chemicals and to be well tolerated by biological tissues following intradermal or subcutaneous administration (1). For these reasons, dimethylpolysiloxane and other types of silicone rubber have found wide application as tissue prostheses and, more recently, as an experimental means of sustained-release drug administration. In 1965, Bass *et al.* (2) demonstrated a prolonged pharmacological response following the subcutaneous administration of atropine encapsulated in segments of silicone rubber tubing. Dziuk and Cook (3) reported that subcutaneous administration of melengestrol acetate encapsulated in silicone rubber tubing or sheeting was a suitable method for the inhibition of estrous in ewes. Numerous *in vitro* studies (3–5) demonstrated that a variety of steroids are able to diffuse through silicone rubber barriers.

Chang and Kincl (6) compared the effectiveness of megestrol acetate as a function of the route of administration. They observed that subcutaneous implantation of segments of silicone rubber tubing containing megestrol acetate was the most efficacious mode of administration; from 6 to 25 times less steroid was needed to produce comparable biological effects. Later, Chang and Kincl (7) presented further evidence indicating that the effectiveness of subcutaneous administration of steroids encapsulated in silicone rubber is much greater than conventional subcutaneous administration of steroids in oil.

A review of this literature suggested that a modification of these procedures might be a convenient method of drug administration for the production of experimental hypertension in rats. Studies are currently in progress in this laboratory to evaluate the antihypertensive activity of a series of grayanotoxin analogs. To evaluate antihypertensive activity properly, it is necessary to conduct the studies in hypertensive animals. Hypertension may be produced in rats by a variety of procedures. One convenient procedure involves unilateral nephrectomy, chronic administration of desoxycorticosterone acetate, and maintenance of