EDGEWOOD ARSENAL
TECHNICAL REPORT

EATR 4002

Synthesis and Isolation of Tetrahydrocannabinol Isomers

by

Richard L. Hively
Friedrich W. Hoffmann

July 1966

Chemical Research Laboratory
Research Laboratories
US ARMY EDGEWOOD ARSENAL
EDGEWOOD ARSENAL, MARYLAND 21010

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SYNTHESIS AND ISOLATION OF TETRAHYDROCANNABINOL ISOMERS

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Project 1C522301A060

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FOREWORD

The work described in this report was performed under Project 1C522301A060, Chemical Agents (U). The work was started in January 1963 and completed in March 1965. The experimental data are contained in notebooks 6958 and 7010.

In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society of Medical Research.

Acknowledgments

All marijuana samples used in this investigation were obtained from Dr. Melvin Lerner, US Customs Laboratory, Baltimore, Maryla l. The sample of Egyptian hashish was made available by Lord Todd, University of Cambridge, England. The authors wish to express their gratitude to Dr. Lerner and Lord Todd for their generous gifts.

DIGEST

In addition to cannabinol, cannabidiol, and trans-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 8, 10a-tetrahydro-6-dibenzopyran (tetrahydrocannabinol A), a new marijuana constituent, trans-1-hydroxy-3-n-amyl-6,6,9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyran (tetrahydrocannabinol B), was isolated from Maryland and Mexican marijuana. Traces of tetrahydrocannabinol B were also found in Egyptian hashish. West Virginia marijuana contained only cannabidiolic acid. A second sample of Mexican marijuana furnished only tetrahydrocannabinol A and cannabinol, while a Spanish sample contained an additional amount of cannabidiol. The structure of tetrahydrocannabinol B was elucidated by chemical and spectral evidence. The partial syntheses of four isomeric tetrahydrocannabinols (A, B, and their cis-isomers) and the total synthesis of the racemic cis-isomer of tetrahydrocannabinol B are also described.

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SYNTHESIS AND ISOLATION OF TETRAHYDROCANNABINOL ISOMERS

I. INTRODUCTION.

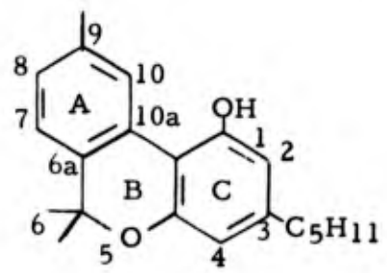
This investigation was undertaken to isolate the constituents of the phenolic fraction from marijuana in the pure state and to determine their identity and structures where unknown. A second objective was the possible synthesis of a tetrahydrocannabinol isomer identical to the natural product.

The flowering tops of the female hemp plant, Cannabis sativa, produce a greenish resin that greatly affects the central nervous system of man. The intoxicating properties of the Cannabis resins have been known for many centuries. Chemical investigations on these resins were started over a century ago, but progress was relatively slow until the 1930's.

In 1847, T. Smith and H. Smith[1] proved that the intoxicating properties of the Cannabis resins were not caused by compounds that contain a basic nitrogen atom. Wood, Spivey, and Easterfield[2] isolated in 1897 the first pure compound from a Cannabis resin, its crystalline acetate. This acetate yielded a yellow resin on hydrolysis, which these investigators named cannabinol.

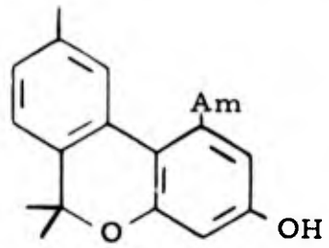
Czerkis,[3] Fraenkel,[4] Casparis,[5] and Bergel[6] in following years tried to isolate cannabinol, but their attempts were unsuccessful. In 1930, Cahn[7] again obtained this compound from a resin extracted from Cannabis indica. Cahn demonstrated by oxidative-degradation studies that cannabinol was a hydroxy-n-amyl-6, 6, 9-trimethyl-6-dibenzopyran.

The structure determination of cannabinol was completed in 1940 independently by Adams and coworkers[8-13] in the United States and by Todd and coworkers[14-16] in England. Both groups of investigators proved by synthesis that the cannabinol is 1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6-dibenzopyran (1).



(1)

The synthetic methods used by Adams and by Todd were very similar; only the Adams method is presented here. Although Cahn's degradation work had rigorously established the configuration of ring A, the positions of the n-amyl group and hydroxyl group in ring C were not known. Adams[9] was able to reduce the possibilities to two alternatives by structurally relating cannabinol to a second phenolic compound[17] that he had isolated from a Cannabis resin. This compound, $C_{21}H_{30}O_2$, was shown by degradation to contain an olivetol (3, 5-dihydroxy-n-amylbenzene) moiety. The similarity between the formulas of cannabinol, $C_{21}H_{26}O_2$, and the second phenolic material, $C_{21}H_{30}O_2$, and the fact that both were derived as natural products from the same source led Adams to postulate that cannabinol might also contain an olivetol moiety. Two structures, (1) and (2), with a 1,3,5- arrangement of the oxygen atoms and the amyl group were possible for cannabinol.

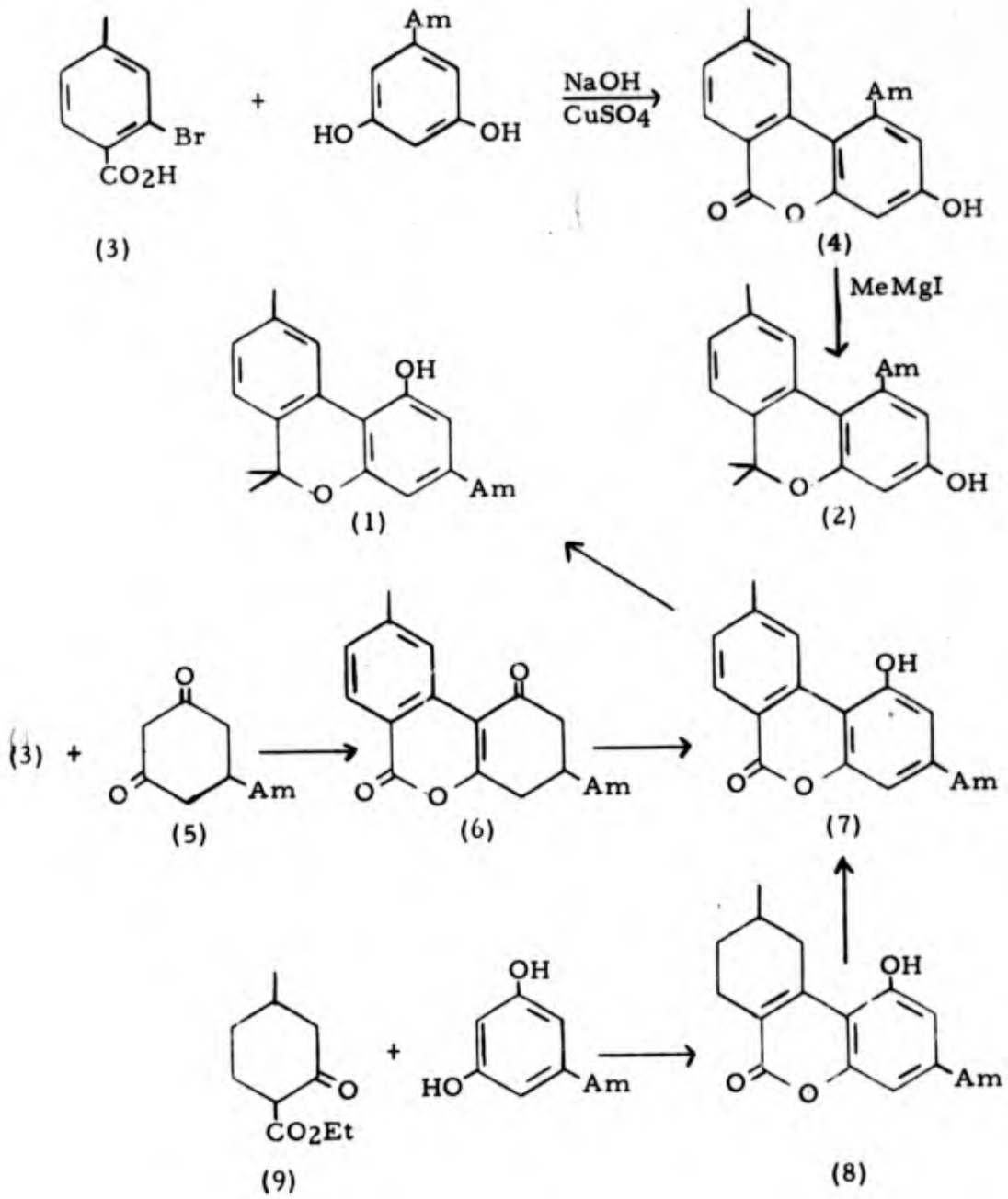


(2)

Adams and coworkers[9] synthesized (2) by the condensation of 4-methyl-2-bromobenzoic acid (3) with olivetol in the presence of dilute aqueous alkali and copper sulfate to form the dibenzopyrone (4). Treatment of this dibenzopyrone with excess methylmagnesium iodide yielded (2). Comparison of (2) with an authentic sample of cannabinol showed that the two compounds were not identical.

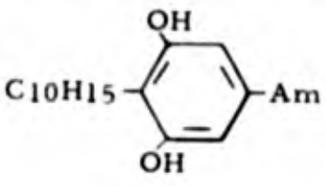
The isomeric compound (1) was synthesized by Adams and coworkers by two methods. The condensation of dihydroolivetol (5) with 4-methyl-2-bromobenzoic acid in the presence of base and copper sulfate[11] yielded the tetrahydropyrone (6), which was dehydrogenated with sulfur to the dibenzopyrone (7). Treatment of (7) with excess methylmagnesium iodide yielded the dibenzopyran (1), which was identical to an authentic sample of cannabinol.

The alternate route employed[13] for the synthesis of (1) consisted of the condensation of ethyl 5-methylcyclohexanone-2-carboxylate (9) with olivetol to form the tetrahydropyrone (8). The sulfur dehydrogenation of (8) yielded the dibenzopyrone (7), which, when methylated with excess methylmagnesium iodide, yielded (1).

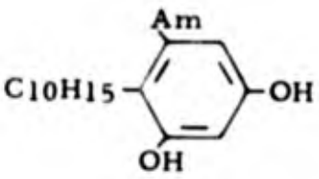

Am
Br
CO2H
+
HO
OH
NaOH
CuSO4
Am
OH
O
O
(3)
(4)
MeMgI
OH
Am
O
Am
(1)
Am
OH
O
(2)
(3) +
O
O
Am
(5)
O
O
O
Am
(6)
OH
O
O
Am
(7)
O
CO2Et
(9)
+
OH
HO
Am
OH
O
O
Am
(8)

Adams and coworkers[17] isolated a second pure constituent, termed cannabidiol, from the flowering tops of Minnesota wild hemp as its crystalline bis(3, 5-dinitrobenzoate). Treatment of this ester in a sealed tube with anhydrous ammonia in toluene yielded a crystalline dihydric phenol, $C_{21}H_{30}O_2$. Jacob and Todd[16] had also isolated cannabidiol from Egyptian hashish but found that cannabidiol was present only in trace amounts in Cannabis resins of Indian origin.

Adams and coworkers[18] identified cannabidiol as a resorcinol derivative on the basis of its infrared (IR) and ultraviolet (UV) spectra. The oxidative degradation of cannabidiol with basic potassium permanganate resulted in the formation of caproic acid, which established the presence of a n-amyl group. From these data, two possible partial structures, (10) and (11), for cannabidiol were suggested by Adams.



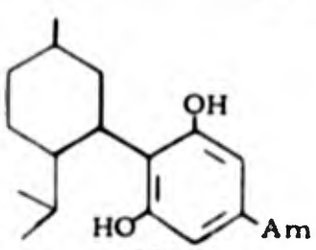
(10)



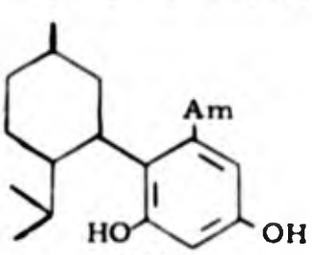
(11)

When cannabidiol was reduced in glacial acetic acid with hydrogen in the presence of a platinum catalyst, 2 moles of hydrogen were absorbed.[16,19] Since the amylresorcinol moiety was unaffected by the catalytic reduction, the $C_{10}H_{15}$ residue was monocyclic and contained two double bonds. Treatment with pyridine hydrochloride at 210° to 230°C resulted in the degradation of cannabidiol to p-cymene and olivetol.[19] The formation of p-cymene proved that the $C_{10}H_{15}$ residue of cannabidiol is a menthyl moiety containing two double bonds.

Oxidation[19] of tetrahydrocannabidiol yielded a menthanecarboxylic acid identical to the menthanecarboxylic acid obtained by the treatment of menthylmagnesium chloride with carbon dioxide. This proved that the olivetol moiety is attached to the $C_{10}H_{15}$ residue adjacent to the carbon bearing the isopropyl group. On the basis of these data, Adams assigned two possible structures, (12) and (13), to the tetrahydroderivative of cannabidiol.

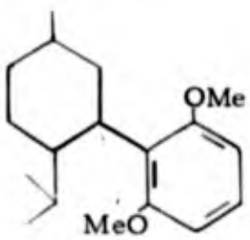


(12)

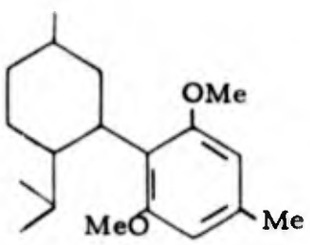


(13)

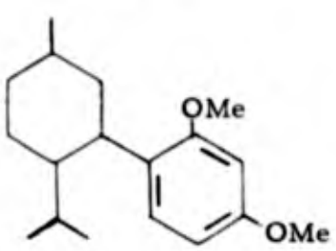
The position of the linkage between the menthyl and the olivetol groups in cannabidiol was established by UV spectroscopy. Adams and coworkers[20] synthesized the model compounds, 2-(3-menthyl)-1,3-dimethoxybenzene (14), 2-(3-menthyl)-1,3-dimethoxy-5-methylbenzene (15), 4-(3-menthyl)-1,3-dimethoxybenzene (16), and 4-(3-menthyl)-1,3-dimethoxy-5-methylbenzene (17), and compared their UV spectra with that of tetrahydrocannabidiol dimethyl ether.



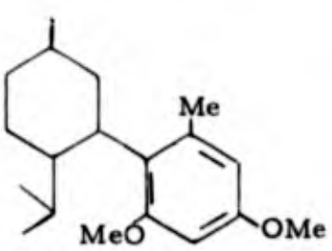
(14)



(15)



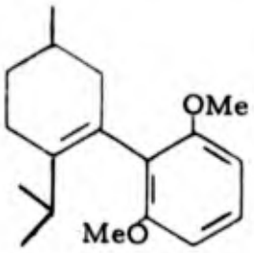
(16)



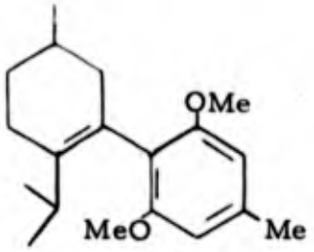
(17)

The UV spectrum of tetrahydrocannabidiol was similar to the spectra of (14) and (15), but differed from those of (16) and (17). From these observations, Adams[20] concluded that the menthyl group in tetrahydrocannabidiol dimethyl ether was linked to the olivetol moiety between the two methoxy groups. Therefore, structure (12) was assigned to tetrahydrocannabidiol.

The structural features of cannabidiol that remained to be established were the positions of the two double bonds in the menthyl residue of cannabidiol. Adams and coworkers attacked this problem by chemical means and by UV spectroscopy. The condensation of 2-lithioresorcinol dimethyl ether and 2-lithioorcinol dimethyl ether with menthone followed by dehydration yielded compounds (18) and (19),[21] respectively.

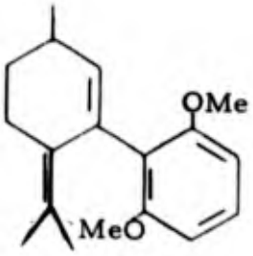


(18)

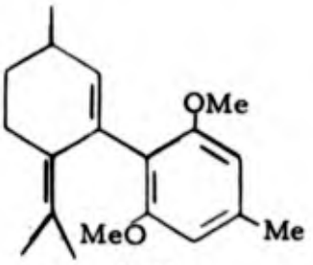


(19)

The UV spectra of (18) and (19) were entirely different from that of the dimethyl ether of natural cannabidiol. On the other hand, the products (20) and (21), obtained from the reaction of 2-lithioresorcinol and 2-lithio-orcinol dimethyl ethers, respectively, with pulegone followed by dehydration, had UV spectra very similar to those of (18) and (19), which contain one double bond in conjugation with the aromatic ring.

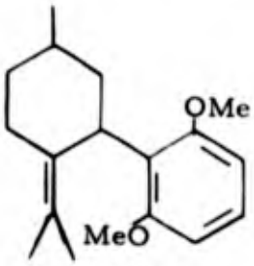


(20)

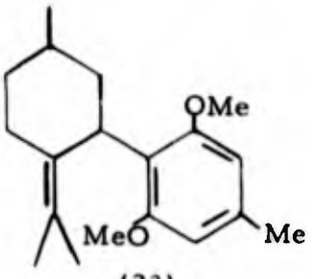


(21)

The spectra of (20) and (21) differed widely, however, from those of cannabidiol dimethyl ether and dihydrocannabidiol dimethyl ether. Partial reduction of (20) and (21) yielded (22) and (23).



(22)

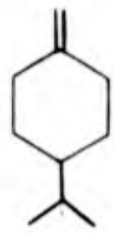


(23)

The UV spectra of (22) and (23) were similar to the UV spectra of cannabidiol dimethyl ether and dihydrocannabidiol dimethyl ether. Since the UV spectra of cannabidiol dimethyl ether and dihydrocannabidiol dimethyl

ether differed from those of the unreduced synthetic compounds (18), (19), (20), and (21) but were similar to the spectra of the synthetic (14), (15), (22), and (23), Adams[21] concluded that the double bonds in cannabidiol are not conjugated with the phenyl nucleus. Jacob and Todd[16] independently reached the same conclusion from the UV absorption spectrum of cannabidiol.

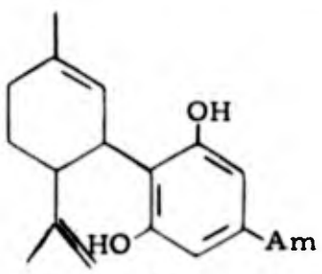
One of the double bonds of cannabidiol was established by Adams as a part of a methylene group by quantitative ozonization to formaldehyde.[21] The two possible positions for the methylene group in cannabidiol are illustrated by the partial structural formulas (24) and (25).



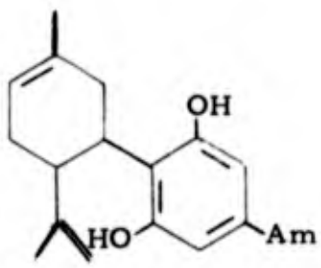
(24)

(25)

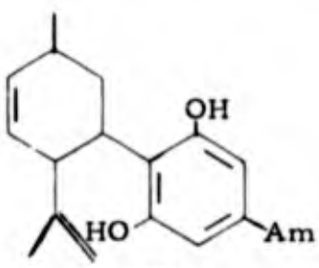
The ring closure of cannabidiol[22] to a tetrahydrodibenzopyran derivative eliminates the exocyclic methylene group of structure (25). These findings limited the structure of cannabidiol to one of the four compounds (26) to (29).



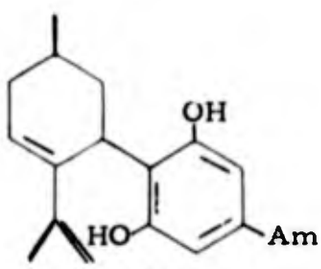
(26)



(27)

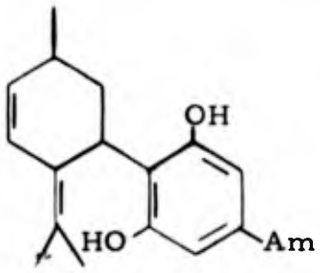


(28)



(29)

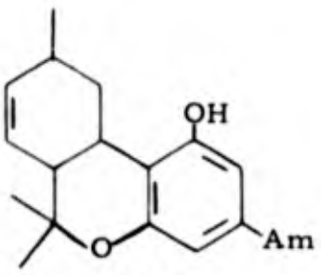
Simonsen and Todd[23] indicated an alternative structure (30) for cannabidiol.



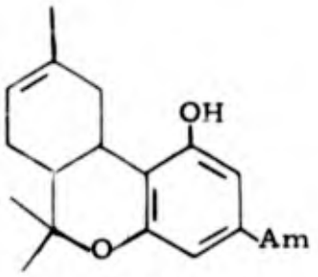
(30)

A strong absorption at 230 mμ (ε 10,000) in the UV spectrum of cannabidiol was interpreted by these investigators as caused by conjugation of the alicyclic double bond of cannabidiol with the exocyclic double bond of the isopropylidene group of (30). They pointed out that the isopropenyl-isopropylidene rearrangement can occur under conditions that suggest that the two structures are in a sense tautomeric. Therefore, ozonolysis of the isopropylidene group sometimes yields formaldehyde rather than the expected acetone. On the basis of the absorption spectrum of cannabidiol and the erroneous results sometimes obtained from the ozonolysis of isopropenyl or isopropylidene groups, Simonsen and Todd indicated that a structure such as (30) for cannabidiol could not be disregarded. The lack of formation of a Diels-Alder adduct from cannabidiol and maleic anhydride and their interpretation of the UV spectrum of cannabidiol led Adams and coworkers[24] to prefer structure (28).

An additional argument for the assignment of structure (28) to cannabidiol was based on the acid-catalyzed cyclization of cannabidiol to two isomeric tetrahydrocannabinols assigned structures (31) and (32) by Adams.[25]



(31)



(32)

The conditions of the cyclization determined which of the two isomers, (31) or (32), was formed. When cannabidiol was refluxed with dilute ethanolic hydrogen chloride, the tetrahydrocannabinol (31) resulted with an optical rotation of $[\alpha]_D$ -130°. A more drastic treatment with p-toluene-sulfonic acid in refluxing benzene yielded the tetrahydrocannabinol (32) with an optical rotation of $[\alpha]_D$ -265°. Treatment of (31) with p-toluenesulfonic acid in refluxing benzene yielded a mixture of (31) and (32) with a maximum rotation of $[\alpha]_D$ -200° to -225°. This led to the assumption that migration of the double bond preceded ring closure. According to Adams,[21] complete conversion of (31) to (32) cannot be achieved because of the increased difficulty of double-bond migration in the cyclized compound.

Since the low-rotating isomer of tetrahydrocannabinol (31) was formed under mild conditions, Adams and coworkers[25] concluded that the alicyclic double bond had not migrated and had the same position as in cannabidiol. The isomer of high rotation (32) was visualized as the product of the double-bond migration to the more stable $\Delta^{8,9}$ position with termination on a tertiary and secondary carbon atom. In the opinion of Adams,[24] the data from the acid-catalyzed cyclization of cannabidiol exclude structures (26) and (29) for cannabidiol. He argued that under the vigorous conditions used in forming the isomer of higher rotation the alicyclic double bond would migrate into the more stable position, conjugated with the phenyl nucleus. Since the UV spectrum of the tetrahydrocannabinol with the higher rotation showed that the alicyclic double bond was not conjugated with the phenyl nucleus, Adams eliminated structures (26) and (29) for cannabidiol.

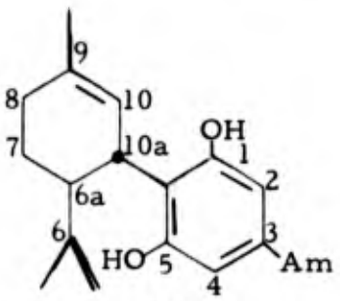
Structure (27) was considered unlikely for cannabidiol by Adams, because of the lack of a driving force for double-bond migration; therefore, only one tetrahydrocannabinol would be obtained by the acid-catalyzed cyclization of a cannabidiol having an alicyclic moiety possessing structure (27).

The fact that the tetrahydrocannabinols (31) and (32) obtained from the acid-catalyzed cyclization of cannabidiol differed only in the position of the alicyclic double bond was shown by the reduction of (31) and (32) to the same hexahydro derivative with a specific rotation of $[\alpha]_D$ -70°. Sulfur dehydrogenation of (31) and (32) yielded cannabinol, which proved the identity of their carbon skeletons.

Two structural features of cannabidiol were not resolved by the studies of Adams and Todd. The exact position of the alicyclic double bond was not rigorously proved by either of these investigators. Another

feature of the cannabidiol molecule not investigated by Adams or Todd was the stereochemical relationship of the isopropenyl and phenyl groups in cannabidiol.

In 1963, Mechoulam and Shvo[26] presented evidence that cannabidiol has the structure represented by formula (33).

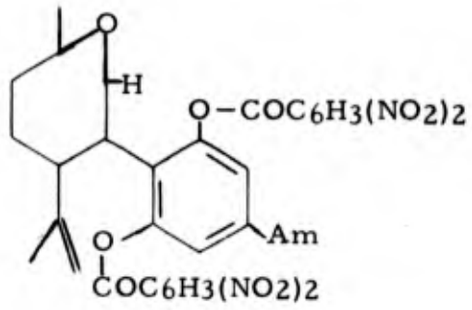


(33)

The position of the double bond was deduced by these investigators from the NMR spectrum of cannabidiol. The NMR spectrum of cannabidiol showed the presence of only three olefinic protons. Two of these were due to the terminal methylene of the isopropenyl group. The third olefinic proton must then be on the alicyclic double bond. This evidence excludes structure (28) proposed by Adams for cannabidiol, since the NMR spectrum of this compound would show the presence of four olefinic protons, two for the terminal methylene and two for the alicyclic double bond.

The presence of only one methyl group on a saturated carbon and two vinylic methyl groups showed that the alicyclic double bond of cannabidiol must be in either the $\Delta^{9,10}$ or $\Delta^{8,9}$ position. Mechoulam and Shvo were able to decide between these possibilities in favor of the $\Delta^{9,10}$ double bond by the change in the signal for the C−10a proton on hydrogenation of cannabidiol. In cannabidiol the signal for the C−10a proton was observed at 3.85 ppm. Since the chemical shift was considered to be too low for a proton α to a phenyl ring only, Mechoulam and Shvo postulated that the C−10a proton must be unshielded by an adjacent double bond. Placing the double bond in the $\Delta^{9,10}$ position satisfied this condition. The $\Delta^{9,10}$ position for the double bond is further supported by the NMR spectrum of tetrahydrocannabidiol. The signal for the C−10a proton at 3.85 ppm in cannabidiol moves upfield to 2.60 ppm in tetrahydrocannabidiol. If the C−10a proton were not allylic to the double bond, hydrogenation of the double bond would have no appreciable effect on the chemical shift of the C−10a proton.

Mechoulam and Shvo[26] added more evidence for the $\Delta^{9,10}$ position of the alicyclic double bond in cannabidiol by the epoxidation of cannabidiol bis(3,5-dinitrobenzoate) to the monoepoxide of structure (34). The NMR spectrum of (34) showed that only the ring double bond had been attacked. A sharp singlet was observed at 3.01 ppm for a proton α to an epoxide oxygen.



(34)

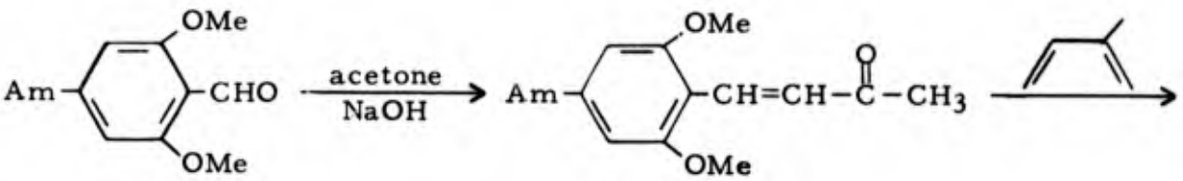
The absence of splitting for the proton α to the epoxide oxygen was explained by the following arguments. The coupling constant between protons on adjacent carbon atoms is a function of the dihedral angle between the protons and is small for a dihedral angle of 70° to 110°. Second, the proton α to an epoxide oxygen of epoxides fused to a rigid cyclohexane ring system forms angles with the adjacent methylene protons so that only one of the methylene protons is coupled with the proton α to the epoxide oxygen. Since Mechoulam and Shvo[26] observed an unsplit signal for the α proton at 3.01 ppm, they concluded that this proton was adjacent to only one proton. This condition is only satisfied by the $\Delta^{9,10}$ position for the double bond of cannabidiol.

Mechoulam and Shvo reported that neither acidic nor basic treatment of cannabidiol caused an isomerization of the $\Delta^{9,10}$ double bond into conjugation with the phenyl nucleus and concluded that this may be due to either hyperconjugation or conformational factors. Since there are numerous bulky substituents in the vicinity of the carbon-carbon bond between the phenyl nucleus and the alicyclic ring, they concluded that a double bond in the alicyclic ring conjugated to the phenyl ring would not be in the same plane as the phenyl, and the driving force for an isomerization leading to conjugation with the phenyl ring would be reduced.

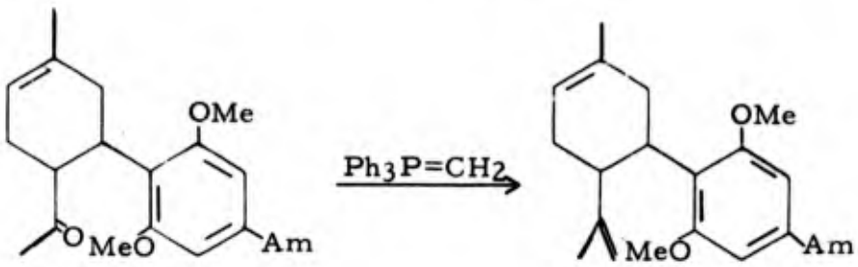
Mechoulam and Shvo[26] proved by means of an equilibration experiment that in cannabidiol the phenyl ring and isopropenyl group are in a trans-relationship to each other. The menthanecarboxylic acid, obtained previously by Adams[19] from the permanganate oxidation of tetrahydrocannabidiol, was identical to the acid obtained by the treatment of menthylmagnesium chloride

with carbon dioxide. Mechoulam and Shvo[26] refluxed a sample of the methyl ester of a menthanecarboxylic acid prepared from menthylmagnesium chloride and carbon dioxide with sodium methoxide in benzene or absolute methanol and obtained only unchanged starting material. They concluded that the menthanecarboxylic acid from tetrahydrocannabidiol was thermodynamically the most stable form in which the isopropyl group and carboxyl group are both equatorial and, therefore, trans to each other. Assuming that no isomerization occurs under the mild oxidation conditions, the orientation of the phenyl nucleus and the isopropenyl group of cannabidiol must both be equatorial and therefore trans.

The synthesis of the $\Delta^{8,9}$-isomer of cannabidiol dimethyl ether has been reported by Hackel.[27]

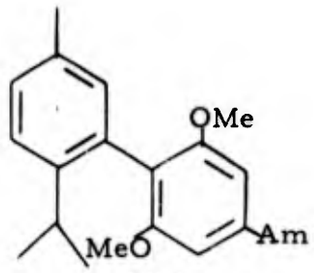


(35) (36)



(37) (38)

The dimethyl ether of olivetol aldehyde (35) was condensed with acetone to form the benzalacetone derivative (36). A Diels-Alder reaction of (36) with isoprene yielded 5-(2, 6-dimethoxy-4-n-amylphenyl)-4-acetyl-1-methylcyclohexene (37), which, when treated with triphenylphosphine methylene, yielded (38). The compound (38) was not identical to an authentic sample of cannabidiol dimethyl ether, but the dehydrogenation of (38) and of cannabidiol dimethyl ether yielded the same product, 2-(2, 6-dimethoxy-4-n-amylphenyl)-4-methyl-1-isopropylbenzene (39), demonstrating that the carbon skeletons of the two compounds were identical.

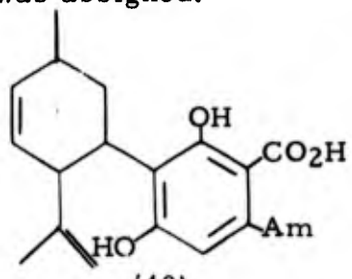


(39)

The presence of an acidic fraction in Cannabis extracts was first observed by Fulton.[28] Fulton extracted a petroleum ether solution of undistilled Cannabis resin from American marijuana with aqueous sodium hydroxide to yield an acidic fraction that was separated into two components by extraction of the basic solution with ether. The ether solution yielded a compound that gave color tests characteristic of cannabinol. After acidification and extraction of the basic solution with ether, a second compound was isolated that gave color tests characteristic of cannabidiol.

Todd and coworkers[29] repeated the work of Fulton on a sample of American marijuana. They isolated the same two components. The component that could be extracted from the basic solution yielded cannabinol when refluxed with methanol or with an aqueous sodium hydroxide solution. The second component that was isolated after acidification of the basic extract yielded cannabidiol when refluxed with methanol or aqueous sodium hydroxide. Todd interpreted these results to indicate that cannabinol and cannabidiol existed in this sample of marijuana as esters of a phenolic acid. He was not, however, able to isolate any such acid from the hydrolysis of the base-soluble components.

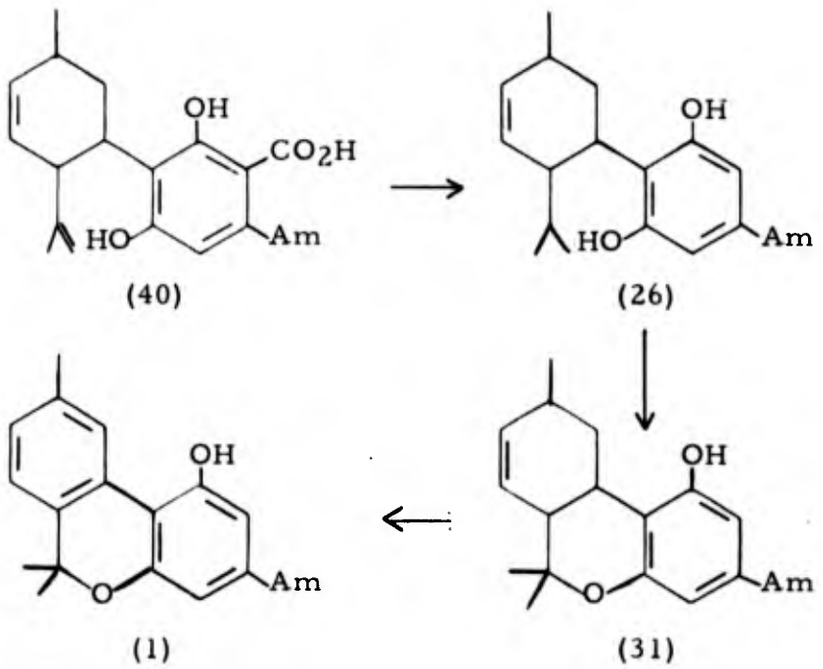
Krejci and Santavy[30-32] in 1955 and Schultz and Haffner[33] in 1958 isolated a base-soluble fraction from unripe Cannabis sativa. By IR and UV spectroscopy and by chemical means, both groups of investigators showed that the base-soluble substance named cannabidiolic acid is a β-resorcylic acid derivative. Schultz and Haffner[33] showed that this acid readily decarboxylates to form cannabidiol (33). Since the decarboxylation of cannabidiolic acid occurs under mild conditions, no isomerization would be expected to occur. To the acid, recognized as a β-resorcylic acid derivative, structure (40) was assigned.



(40)

Schultz and Haffner[33] showed that cannabidiolic acid had a sedative action in mice at dose levels of 0.05 to 0.1 mg/gm and an LD50 value of 0.25 to 0.5 mg/gm. The acid does not produce the euphoric effect characteristic of hashish,[33] but was shown by Krejci and Santavy[34] to exhibit an antibiotic effect on certain microorganisms.

Schultz and Haffner[35] postulated that cannabidiolic acid is the precursor of the other phenolic compounds of related structure found in Cannabis extracts.



Scheme I

The biogenetic mechanisms by which these conversions take place are not known. According to Farmilo,[36] the abundance of the four compounds is in the order of (40)>(26)>(31)>(1). The factors that influence the amounts of the compounds found in extracts of various Cannabis specimens are climatic conditions under which the plants are grown, soil conditions, and plant variety.

Schultz and Haffner[35] conducted experiments in which seeds from various origins were grown at the Max Planck Institute for Agricultural Research in Hamburg, Germany, in the same field under identical conditions and for the same length of time. It was found that for northern varieties of

Cannabis the amounts of (26) and (31) were low, but the amount of cannabidiolic acid present was in the range of 78% to 83% of the total resin. The varieties from tropical climates were lower in cannabidiolic acid (56% of the total resin) and higher in content of (1), (26), and (31). Varieties from Mediterranean climates had cannabidiolic acid contents that were intermediate between the northern and tropical varieties.

According to Schultz and Haffner[35] and Farmilo,[36] the temperature of northern climates may be the factor that inhibits the action of the enzymes responsible for the production of (1), (26), and (31). Schultz and Haffner showed that temperature is a factor that affects the amount of the four compounds (1), (26), (31), and (40) present in Cannabis extracts. These investigators showed that heat causes the conversion of cannabidiolic acid to the other phenolic substances found in the Cannabis plant (table I).

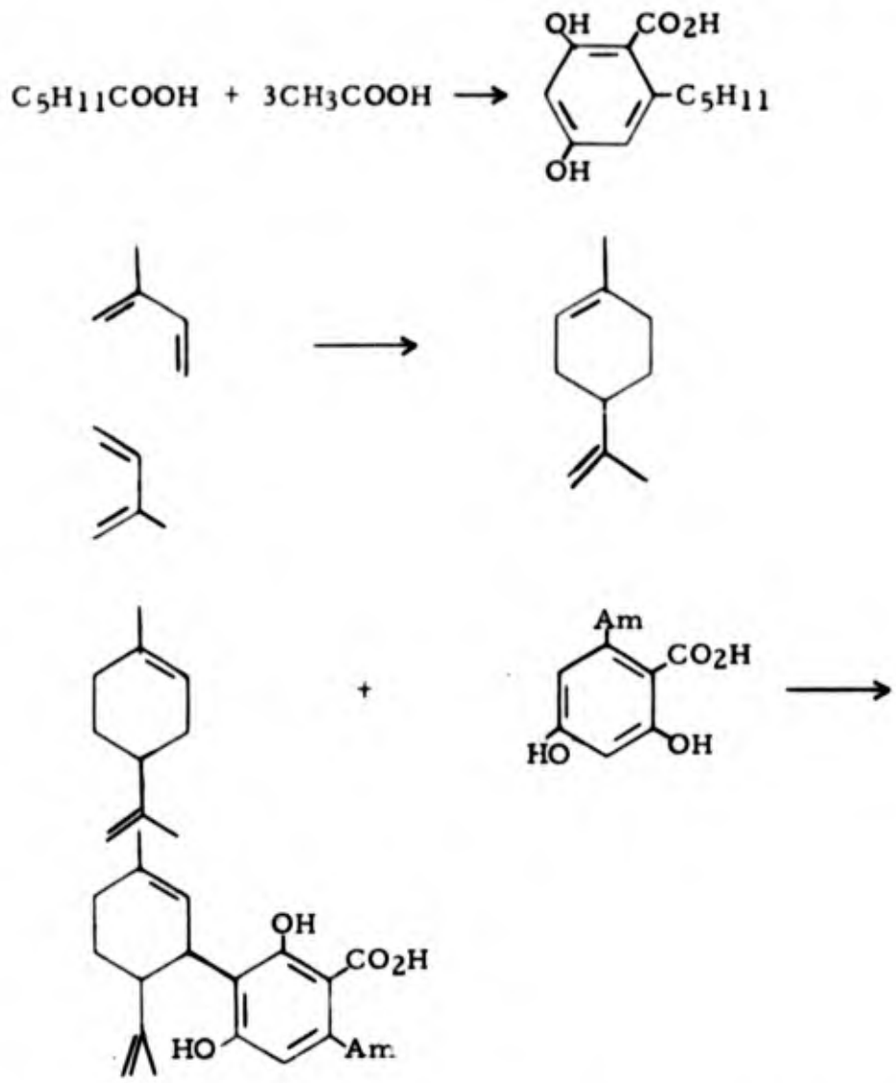
Table I. The Effect of Heat on the Composition of Cannabis Resin From Cannabis fibridia

Period of heating	Cannabidiolic acid	Cannabidiol	Tetrahydrocannabinol and cannabinol
hr		gm %	
0	1.52	0.139	0.252
24	0.72	1.1	0.39
36	0.53	1.0	0.42
48	0.41	0.98	0.548

Upon heating, the amount of cannabidiolic acid initially decreases rapidly with a large increase of cannabidiol content and a small increase in the amount of tetrahydrocannabinol and cannabinol. When the heating was continued, the cannabidiolic acid content decreased slowly and the content of cannabidiol became almost constant, while the amount of tetrahydrocannabinol and cannabinol increased slowly.

Cannabidiolic acid appeared from the work of Schultz and Haffner[35] to be the primary biogenetic product of the Cannabis plant and the precursor of the other phenolic constituents. Todd[23] had suggested before the discovery of cannabidiolic acid that cannabidiol was the primary plant

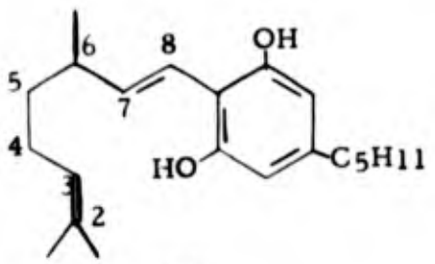
product. He suggested that cannabidiol could be formed by the condensation of olivetol and a menthatriene. The isolation from a Cannabis extract of p-cymene and 4-methylisopropenylbenzene, which could easily arise from a menthatriene, appeared to strengthen Todd's conclusion. The discovery of the cannabidiolic acid led Farmilo[36, 37] to postulate for the biogenetic synthesis of the Cannabis constituents a new hypothetical pathway as outlined in the following scheme.



The remainder of the transformations to form the other Cannabis constituents are illustrated by scheme I.

The above biogenetic scheme has recently been placed in doubt by the isolation of a new hashish constituent by Gaoni and Mechoulam.[38] This material, cannabigerol, is closely related to cannabidiol; its UV spectrum is

identical to that of cannabidiol. Cannabigerol has the empirical formula $C_{21}H_{32}O_2$, compared to $C_{21}H_{30}O_2$ for cannabidiol. Cannabigerol is optically inactive but has the same number of double bonds as cannabidiol. These data led Gaoni and Mechoulam to postulate that the carbon-carbon bond between the two asymmetric centers in cannabidiol does not exist in cannabigerol. The NMR spectrum of cannabigerol showed the presence of two equivalent aromatic protons, a methylene group (C-8) strongly deshielded and split by a single proton, and two olefinic protons. These data indicated a structure represented by (41) for cannabigerol.



(41)

Cannabigerol (41) was prepared synthetically by refluxing geraniol and olivetol in decalin for 36 hr.

Gaoni and Mechoulam[38] believe that cannabigerol is probably formed in nature by the condensation of olivetol with geranyl pyrophosphate and that, from a biogenetic point of view, cannabigerol represents the primary biogenetic product of the Cannabis plant and a missing link in the formation of the Cannabis constituents.

The most important constituent of hashish and "red oil" is tetrahydrocannabinol, which is responsible for the characteristic euphoric effects in man.[39,40] Jacob and Todd[16] isolated a small amount of a substance that appeared to be a tetrahydrocannabinol but could not be completely identified because of a lack of material. Haagen-Smit and coworkers[41,42] isolated a crystalline compound, mp 128°C, from a distilled Minnesota wild-hemp extract. The physiological activity of this compound was much higher than that of the distilled or crude extract and differed from that of cannabinol and cannabidiol. Haagen-Smit and coworkers[41] did not obtain enough of this substance to establish its chemical identity but believed that it may have been a tetrahydrocannabinol.

Wollner, Matchett, Levine, and Loewe[43] isolated a tetrahydrocannabinol acetate from Indian charas by chromatography on alumina and vacuum distillation. Four of the fractions from the high-vacuum distillation

of the chromatographed tetrahydrocannabinol acetate had a constant refractive index and optical rotation. On the basis of this finding, these investigators assumed the material to be a homogeneous tetrahydrocannabinol. It showed a high physiological activity as manifested by the response in the dog ataxia test.

Hydrolysis of the tetrahydrocannabinol acetate was accomplished by acid, alkali, or ammonia in alcoholic solutions. The hydrolysis product in all instances showed a reduced physiological activity and a change in specific rotation from $[\alpha]_D$ -214° for the acetate to $[\alpha]_D$ -193° for the hydrolysis product. Reacetylation of the hydrolysis product did not restore the physiological activity or the optical rotation to their initial values. These data indicated that an isomerization had occurred during the hydrolysis.

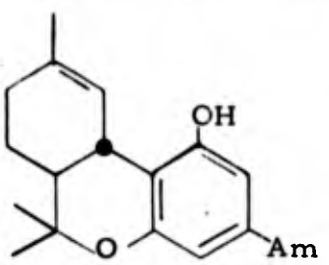
Korte and Sieper[44] isolated a small amount of a crystalline tetrahydrocannabinol, mp 120° to 125°C, from German hemp by countercurrent distribution. This compound may have been identical to the tetrahydrocannabinol, mp 128°C, isolated by Haagen-Smit and coworkers.[41,42]

Powell and coworkers[45] isolated a crystalline 3,5-dinitrophenylurethan from a Cannabis extract. The decomposition melting point of this material was intermediate between that of the 3,5-dinitrophenylurethans of cannabidiol and cannabinol. Hydrolysis yielded a material with a physiological activity two or three times greater than that of the standard synthetic tetrahydrocannabinol (44), but only one-fifth to one-third as active as the Cannabis extract from which the compound was derived. The material was presumably an impure tetrahydrocannabinol. This was indicated solely on the basis of the physiological activity of the compound.

In 1960, DeRopp[46] developed a partition-chromatographic system for the separation of the Cannabis constituents. By the use of N,N-dimethylformamide as the stationary phase on Celite and cyclohexane saturated with N,N-dimethylformamide as the mobile phase, DeRopp isolated a tetrahydrocannabinol from the methanol extract of Mexican marijuana. This material was a colorless resin that was a solid at room temperature and showed a high physiological activity in dogs. The UV spectrum of the tetrahydrocannabinol isolated by DeRopp was in agreement with the UV absorption data Wollner and coworkers[43] had obtained for the tetrahydrocannabinol isolated from Indian charas.

Recently, Gaoni and Mechoulam[47] reported the isolation, partial synthesis, and structure determination of an active constituent of hashish. The tetrahydrocannabinol was isolated by chromatography on Florisil and alumina. Further purification of the compound was accomplished by conversion

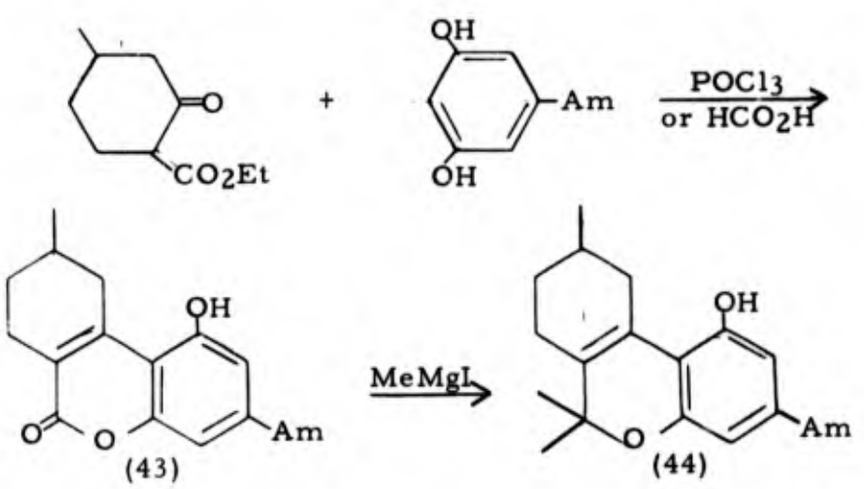
to the crystalline 3,5-dinitrourethan derivative. Mild hydrolysis of the urethane yielded a pure tetrahydrocannabinol that gave cannabinol (1) by sulfur dehydrogenation defining its carbon skeleton. Gaoni and Mechoulam[47] assigned structure (42) to this tetrahydrocannabinol on the basis of its NMR spectrum, which showed the presence of one aliphatic methyl group and three methyl groups either α to an oxygen atom or substituted on an unsaturated carbon atom. This placed the double bond in either the $\Delta^{9,10}$ or $\Delta^{8,9}$ position. A signal for an olefinic proton was observed at 6.35 ppm in (42), compared with 5.50 ppm for the olefinic proton of cannabidiol (33). The interpretation given by Gaoni and Mechoulam for these data was that the pyran ring of tetrahydrocannabinol tilts the aromatic ring into the same plane as the olefinic proton. The ring currents of the phenyl ring cause the olefinic proton to be unshielded and shifted downfield. Such an effect could only occur if the alicyclic double bond of (42) were in the $\Delta^{9,10}$ position and the protons of the 6a and 10a carbon atoms were trans to each other.



(42)

The same compound was prepared by Gaoni and Mechoulam[47] by the treatment of cannabidiol with 0.05% hydrochloric acid in refluxing ethanol.

The first total synthesis of a tetrahydrocannabinol was performed by Adams and coworkers[48] and by Todd and coworkers[49] independently.



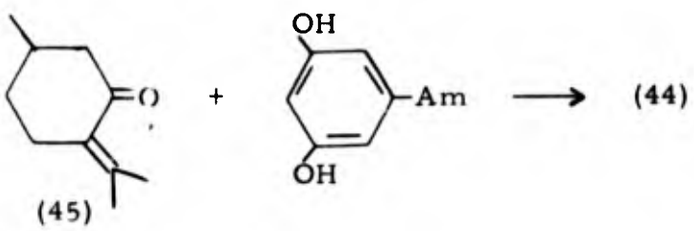
(43) (44)

Olivetol was condensed with ethyl 5-methylcyclohexanone-2-carboxylate in the presence of phosphorus oxychloride, and the resulting pyrone (43) was treated with excess methylmagnesium iodide to yield 1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-7, 8, 9, 10-tetrahydro-6-dibenzopyran (44). Adams[48] found that (44) had a physiological activity of about one-seventh that found for the tetrahydrocannabinols obtained from the acid-catalyzed cyclization of cannabidiol. He reported that (44) was a colorless viscous resin after purification by high-vacuum distillation. Korte and Sieper[44] repeated the Adams' preparation and, by subjecting the resin obtained from the vacuum distillation to further purification by countercurrent distribution, these investigators obtained two crystalline compounds. The most abundant compound had a melting point 62° to 63°C and was assigned structure (44). The second isomer obtained in small quantities had a melting point of 129°C. The UV spectrum of the material melting at 62° to 63°C was identical to the spectrum of the high-melting material. Korte and Sieper proposed that the high-melting isomer differed from the low-melting isomer with regard to the position of the alicyclic double bond.

Adams and coworkers[50] prepared the optically active tetrahydrocannabinols with structure (44) by the condensation of ethyl (+)- and (-)-5-methylcyclohexanone-2-carboxylate with olivetol to yield the optically active pyrones (43), which were converted by treatment with excess methylmagnesium iodide to the optically active tetrahydrocannabinols. Both antipodes of (44) showed physiological activity.

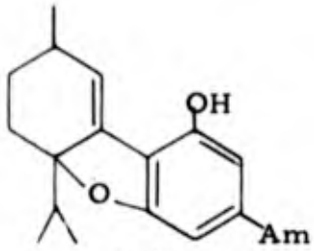
According to Adams and coworkers,[50] the potency of optically active tetrahydrocannabinols is 0.38 and 1.66 for (+)- and (-)-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-7, 8, 9, 10-tetrahydro-6-dibenzopyran, using (d,*l*-44) as the standard.

A second method for the synthesis of (44) was developed simultaneously by both Adams[51] and Todd.[52, 53]

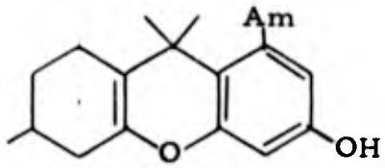


(45)

Pulegone (45) was condensed with olivetol to yield (44). In addition, byproducts of similar structure were formed that made the purification of (44) extremely difficult. Possible structures for some of the byproducts are (46) and (47).



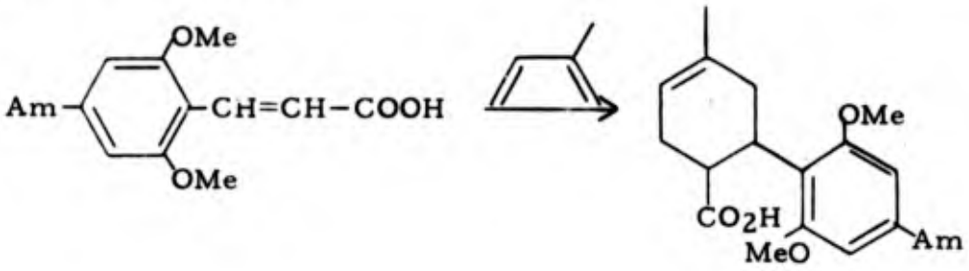
(46)



(47)

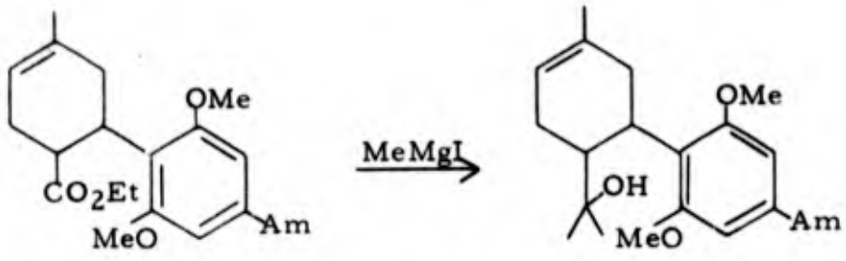
Many analogs and homologs of the synthetic tetrahydrocannabinol (44) have been prepared by several groups of investigators.[48-50, 52-62] While wide variations in potency of these compounds were observed, physiologically more active ones caused the same effects in dogs as (44).

The total synthesis of a tetrahydrocannabinol that has the alicyclic double bond not conjugated with the phenyl nucleus proved to be a difficult task. Adams and Carlin[63] attempted the synthesis of a tetrahydrocannabinol with a $\Delta^{8,9}$ double bond.

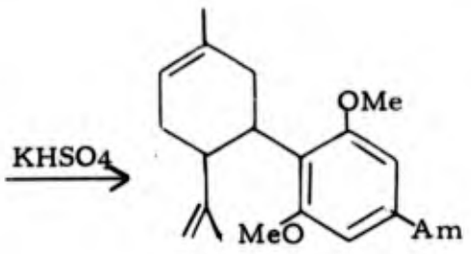


(48)

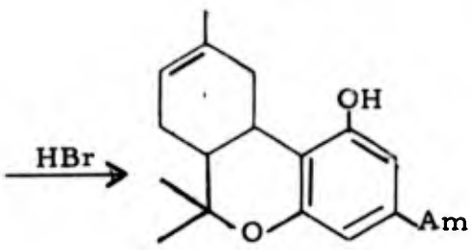
Isoprene was condensed with 2, 6-dimethoxy-4-n-amylcinnamic acid to form (48). Numerous attempts to demethylate (48) to obtain a lactone that when treated with methylmagnesium iodide should yield a tetrahydrocannabinol were unsuccessful. An alternate route tried by Wicks[64] resulted in limited success.



(49) (50)



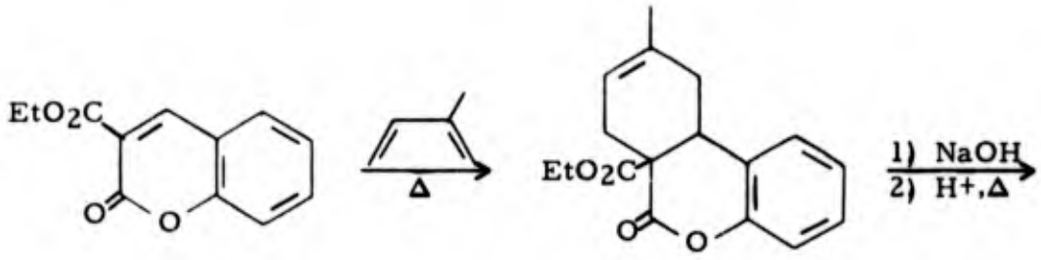
(51)



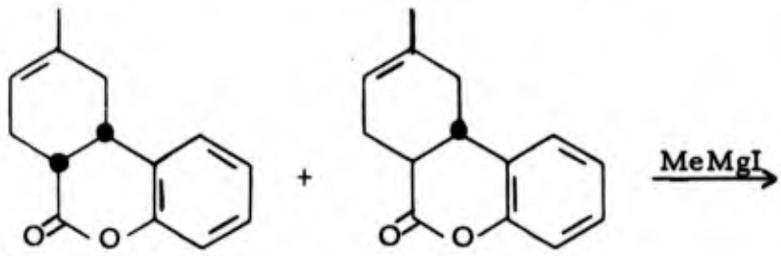
(52)

The acid (48) was esterified and the resulting ethyl ester (49) was treated with 2 moles of methylmagnesium iodide to form (50). Dehydration of (50) with potassium hydrogen sulfate yielded (51), an isomer of cannabidiol dimethyl ether. Treatment of (51) with hydrobromic acid in refluxing acetic acid yielded mostly p-cymene and olivetol, but a small amount of two compounds, each of which had an elemental analysis consistent with that of a tetrahydrocannabinol, was isolated. Little data as to the actual structure of this material were obtained by Wicks. The UV spectra of these compounds did not agree with UV spectrum of the natural tetrahydrocannabinols. The physiological activity of the synthetic materials was about one-half that of the standard (44) and considerably less than the tetrahydrocannabinols of natural origin.

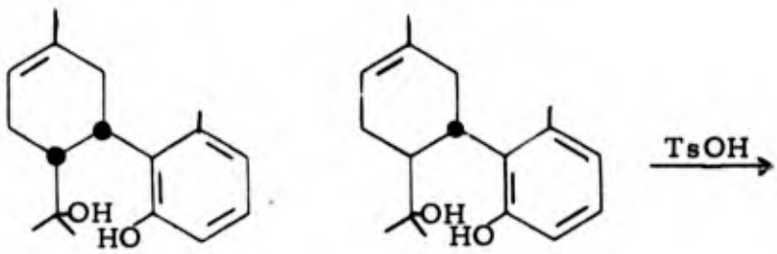
Strojny and Taylor[65] attacked the problem of the synthesis of a tetrahydrocannabinol having an alicyclic double bond not conjugated with the benzene nucleus by condensing isoprene with a coumarin possessing an activated $\Delta^{3,4}$ double bond.



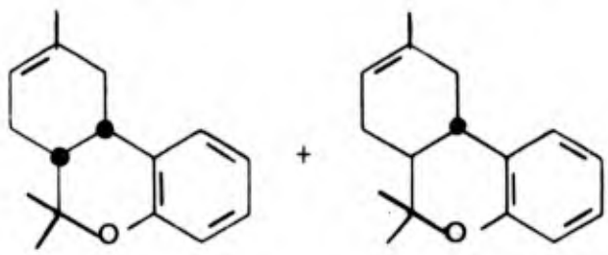
(53) (54)



(55) (56)



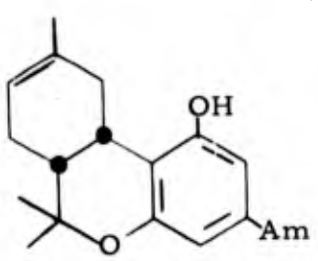
(57) (58)



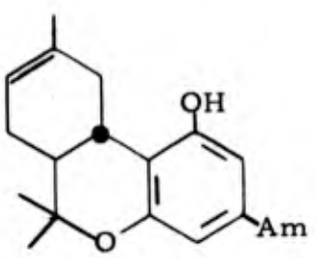
(59) (60)

Isoprene was condensed with 3-carbethoxycoumarin (53, to yield the 6a-carbethoxy-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone (54). Basic hydrolysis of (54) followed by decarboxylation yielded approximately equal amounts of the cis- and trans-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrones (55) and (56). The pyrones (55) and (56) were separated by fractional crystallization and each treated with methylmagnesium iodide to form the cis- and trans-diols (57) and (58), respectively. Treatment of (57) and (58) with p-toluenesulfonic acid in refluxing toluene resulted in the dehydration and cyclization to form the cis- and trans-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrones (59) and (60). Their UV spectra showed that the alicyclic double was not conjugated with the aromatic ring in either instance. The appearance of a new maximum in the UV spectrum of the cis-pyran (59) indicated that some double-bond migration had occurred under the conditions of the dehydration and cyclization. It had not, however, led to conjugation with the phenyl nucleus.

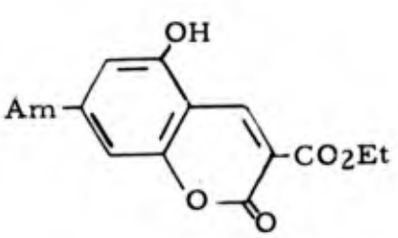
The synthesis of the cis- and trans-tetrahydrocannabinols (61) and (62) having a $\Delta^{8,9}$-alicyclic double bond from (63) or (64) in the same manner as described for the conversion of (53) to (59) and (60) was suggested by Strojny and Taylor.[65]



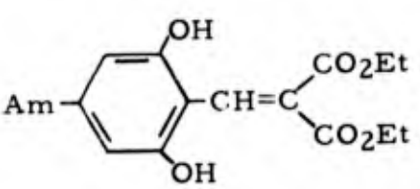
(61)



(62)

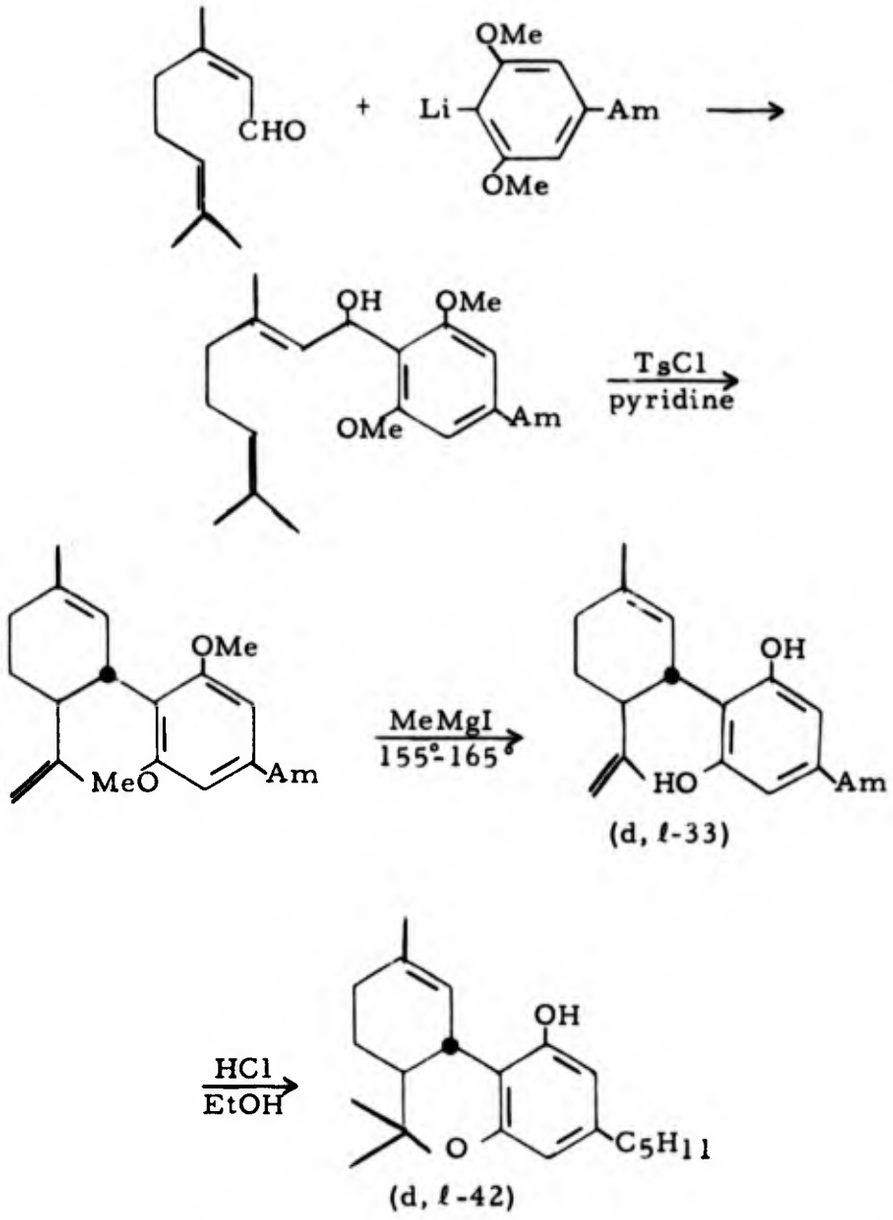


(63)



(64)

Gaoni and Mechoulam[66] have recently reported a total synthesis of both d, ℓ-cannabidiol (33) and d, ℓ-tetrahydrocannabinol (42).



Citral was treated with lithioolivetol dimethyl ether to yield a complex mixture of products.. Treatment of this mixture with p-toluenesulfonyl chloride in pyridine at room temperature yielded cannabidiol dimethyl ether among other products. Demethylation of cannabidiol dimethyl ether was accomplished by heating with excess methylmagnesium iodide at

155° to 165°C for 15 min. The resulting optically inactive cannabidiol (d, ℓ-33) was treated with ethanolic hydrogen chloride to yield (d, ℓ-42). The NMR and IR spectra of d, ℓ-cannabidiol (d, ℓ-33) and d, ℓ-tetrahydrocannabinol (d, ℓ-42) were identical to the spectra of naturally occurring cannabidiol (33) and tetrahydrocannabinol (42).

III. EXPERIMENTATION.

A. Equipment and Material.

The NMR spectra reported in this paper were taken on a Varian Model A-60 spectrometer in deuterochloroform. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as the internal standard.

The IR data reported were determined on a Perkin-Elmer Model 421 spectrometer. The spectra of solids were determined as potassium bromide pellets, and the spectra of liquids were determined as films unless indicated otherwise.

The UV data reported were determined on a Cary Model 14 spectrometer in ethanol.

Melting points were taken on a Hoover oil-bath, melting-point apparatus.

Ordinary laboratory glassware and equipment were used in all isolation procedures and syntheses.

B. Experimentation.

1. Preparation of 3-Acetylcoumarin (65).[67]

A mixture of 32.5 gm (0.25 mole) of ethyl acetoacetate and 30.5 gm (0.25 mole) of o-hydroxybenzaldehyde was cooled to -5°C in an ice-salt bath. A solution of 0.3 gm of piperidine in 2 ml of absolute ethanol was added at such a rate as to maintain the temperature of the reaction mixture at -5°C. After the reaction mixture had stood at -5°C for 12 hr, it had completely solidified. The solid mass was washed with 20 ml of cold ethanol and dried on a Buchner funnel. The bright-yellow solid was crystallized first from ethanol and finally from water to yield 38 gm (81% yield) of pale-yellow needles, mp 123° to 125°C.

Analysis of $C_{11}H_8O_3$:

Calculated: C, 70.2; H, 4.3
Found: C, 69.8; H, 4.4

2. Preparation of 6a-Acetyl-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone (66).

A mixture of 25 gm (0.13 mole) of 3-acetylcoumarin, 17.2 gm (0.26 mole) of freshly distilled isoprene, 25 ml of dry xylene, and 0.01 gm of hydroquinone was sealed in a 500-ml, heavy-walled pyrex tube under a nitrogen atmosphere. The tube was heated with shaking in a steel bomb at 180°C for 18 hr. After cooling in a dry ice-acetone bath, the tube was opened and the contents washed out with benzene. The volatile components were distilled off under reduced pressure. The residue was dissolved in 1 ℓ of hexane and placed on a column packed with 650 gm of silica gel (Mallinckrodt). The column was developed and eluted with a hexane-ether (20:1) solvent system. Cuts of 30 ml were taken from the column at a rate of 2 ml/min. Each cut was slowly reduced in volume under a stream of nitrogen until crystallization began. The concentrates were allowed to stand at room temperature until crystallization was complete. The solids were isolated and combined into fractions on the basis of their melting points. The major fraction contained 16.7 gm of a white crystalline solid, mp 120° to 121°C. A second fraction contained 2.1 gm of a white solid, mp 106° to 108°C. A third fraction containing 2 gm of a white solid melting at 85° to 95°C was a mixture of the major and minor fractions.

Analysis of $C_{16}H_{16}O_3$:

Calculated: C, 74.98; H, 6.29
Found (major fraction): C, 74.9; H, 6.0
Found (minor fraction): C, 74.7; H, 6.3

UV data for major fraction: λ_{max} 275 (ε 742), 268 (ε 814), and 262 mμ (ε 679) in ethanol. IR data: $\nu C{=}O$ 1770 and 1705 cm^{-1}.

3. Hydrolysis of 6a-Acetyl-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone (66).

To a solution of 0.64 gm of sodium hydroxide in 75 ml of water contained in a 200-ml round-bottomed flask equipped with a reflux condenser and magnetic stirrer was added 2 gm (0.078 mole) of (66) in 25 ml of methanol. The solution was heated to reflux for 30 min and then allowed to cool to room temperature. After the methanol had been distilled off under reduced pressure,

a white precipitate formed, which was isolated. The filtrate was acidified with dilute hydrochloric acid and extracted with three 25-ml portions of ether. The ether extract was dried over sodium sulfate and the solvent removed in vacuo. The resulting solid was recrystallized from ethanol to yield 0.8 gm of colorless crystals, mp 143° to 145°C. The IR spectrum of this solid was identical to the spectrum of an authentic sample of trans-9-methyl-6a, 7, 10,-10a-tetrahydro-6-dibenzopyrone (56).

The precipitate that formed after the methanol had been distilled from the reaction mixture was crystallized from ethanol to yield 0.4 gm of (68), colorless crystals, mp 150° to 151°C.

Analysis of $C_{15}H_{18}O_2$:

Calculated: C, 78.23; H, 7.90
Found: C, 78.3; H, 7.8

A 1-gm (0.004-mole) sample of (66) was dissolved in 25 ml of 2% ethanolic potassium hydroxide solution. After the solution stood at room temperature for 3 days, the ethanol was distilled off at 25°C under reduced pressure. The resulting residue was dissolved in 50 ml of water. The aqueous solution was acidified with dilute hydrochloric acid and extracted with three 25-ml portions of ether. After drying over sodium sulfate, the ether was distilled off under reduced pressure. The resulting residue was crystallized from hexane to yield 0.8 gm (93% yield) of a white solid, mp 91° to 93°C. The IR spectrum of this solid was identical to the spectrum of cis-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyran (55).

4. Preparation of cis- and trans-9-Methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrones (55) and (56).

A mixture of 20 gm (0.11 mole) of 3-carboxycoumarin, 17.2 gm (0.26 mole) of freshly distilled isoprene, 25 ml of dry xylene, and 0.01 gm of hydroquinone was sealed under a nitrogen atmosphere in a heavy-walled pyrex tube of 500-ml capacity. The tube was placed in a steel bomb and heated with shaking to 180°C for 18 hr. After cooling in a dry ice-acetone bath, the tube was opened and the contents washed out with benzene. The solvents were distilled from the reaction mixture under reduced pressure. The resulting residue was crystallized from ethanol to yield 16 gm (68% yield) of (55) and (56). Fractional crystallization from ethanol yielded 1.8 gm of (55) and 2.0 gm of (56). The cis-pyrone (55) melted at 94° to 96°C (literature[65] mp 95.5° to 97°C). The trans-pyrone (56) melted at 144° to 145°C (literature[65] mp 143° to 144°C). The mixed melting point of (55) and (56) was 78° to 135°C.

Analysis of $C_{14}H_{14}O_2$:

Calculated: C, 78.48; H, 6.59
Found: (55) C, 78.6; H, 6.5; (56) C, 78.3; H, 6.7

UV maxima: (55) 273 (ε 718), 266 (ε 752), 260 (ε 597), 223 (ε 5140); (56) 274 (ε 708), 267 (ε 776), 222 mμ (ε 5230). IR data: (55) $\nu C{=}O$ 1765 cm^{-1} (solid), $\nu C{=}O$ 1780 cm^{-1}, 5% solution in CCl_4; (56) $\nu C{=}O$ 1750 cm^{-1} (solid), $\nu C{=}O$ 1770 cm^{-1}, 5% solution in CCl_4.

5. Hydrogenation of (55).

A 5-gm (0.023 mole) sample of (55) dissolved in 50 ml of ethyl acetate and 0.1 gm of platinum oxide catalyst were hydrogenated at 3 atm and room temperature for 30 min. The catalyst was filtered from the ethyl acetate solution. The ethyl acetate was distilled off under reduced pressure and the resulting residue dissolved in petroleum ether (30° to 60°C). After cooling, the petroleum ether solution yielded 3.3 gm (66%)[69] of white crystalline solid, mp 74° to 76°C.

Analysis of $C_{14}H_{16}O_2$:

Calculated: C, 77.75; H, 7.46
Found: C, 77.7; H, 7.4

6. Hydrogenation of (56).

A 4.6 gm (0.021 mole) sample of (56) dissolved in 50 ml of ethyl acetate and 0.1 gm of platinum oxide catalyst were hydrogenated at 3 atm for 30 min. After the catalyst was filtered off, the ethyl acetate was removed by distillation under reduced pressure. The colorless residue was fractionally crystallized from hexane to yield two dihydro derivatives, (73a), mp 135° to 137°C, and (73b), mp 65° to 67°C.

Analysis of $C_{14}H_{16}O_2$:

Calculated: C, 77.75; H, 7.46
Found: (73a) C, 77.5; H, 7.6; (73b) C, 77.7; H, 7.4

IR data: (73a), $\nu C{=}O$ 1745 cm^{-1}; (73b), $\nu C{=}O$ 1760 cm^{-1}.

7. Treatment of cis-9-Methyl-6a, 7, 8, 9, 10, 10a-hexahydro-6-dibenzopyrone (71) With Ozone.[68]

A 1-gm sample of (69) was dissolved in 100 ml of glacial acetic acid. Ozonized oxygen was passed through the acetic acid solution at room temperature for 4 hr. After adding 100 ml of 3% hydrogen peroxide solution, the reaction mixture was allowed to stand at room temperature for 12 hr. A white precipitate that formed during this time was removed by filtration. This material (0.5 gm) was identified by its IR spectrum as starting material (69). The solvent was distilled from the filtrate under reduced pressure. The residue was treated with 10 ml of 5% sodium carbonate solution. A small amount of a green resin that was insoluble in the basic solution was removed by filtration. The basic solution was acidified with dilute hydrochloric acid. After the acidified solution had stood in the cold for 1 wk, 0.09 gm of a yellow crystalline solid was deposited. Two recrystallizations from 80% aqueous acetic acid yielded a white crystalline solid melting at 170° to 171°C. The IR spectrum of this solid was identical to the IR spectrum of an authentic sample of cis-4-methyl-cis, cis-hexahydrophthalic acid (70).

8. Treatment of trans-9-Methyl-6a, 7, 8, 9, 10, 10a-hexahydro-6-dibenzopyrone (71) With Ozone.[68]

A 1-gm sample of trans-(71) (= 73a + 73b) was treated in the manner described for the ozonolysis of the cis-(71) to yield 0.18 gm of starting material, a black nonacidic resin, and 5 mg of an acid. The IR spectrum of this acid indicated this material was cis-4-methyl-cis, trans-hexahydrophthalic acid (72). The reaction was repeated several times, but only small amounts of the impure acid could be isolated.

9. Preparation of cis-4-Methyl-cis, cis-hexahydrophthalic Acid (70) and trans-4-Methyl-cis, cis-hexahydrophthalic Acid.[69]

A 10-gm sample of 4-methyl-1, 2, 3, 6-tetrahydrophthalic anhydride, prepared by the addition of isoprene to maleic anhydride, was dissolved in 50 ml of ethyl acetate and hydrogenated at room temperature in a Parr apparatus using platinum oxide as the catalyst. The hydrogenation continued for 2 hr, after which an equivalent of hydrogen had reacted. After the catalyst was filtered off, the ethyl acetate was distilled from the filtrate, and the residue, a colorless oil, was washed with petroleum ether at 0°C to leave a white solid. A 4-gm sample of this solid, mp 32° to 34°C (literature[69] 35° to 36.5°C) was refluxed with 15 ml of water for 20 min. The solution was cooled and the solid

that formed was isolated. Recrystallization of this solid from aqueous methanol yielded 3 gm of cis-4-methyl-cis, cis-hexahydrophthalic acid, mp 170° to 171°C (literature[69] mp 172° to 173°C).

Evaporation of the petroleum ether solution used to wash the hydrogenation product yielded 2.5 gm of a colorless oil. This oil was refluxed with 15 ml of water for 20 min. A white solid precipitated from the water solution on cooling in an ice bath. This solid was crystallized from chloroform to yield 1.5 gm of trans-4-methyl-cis, cis-hexahydrophthalic acid, mp 175° to 176°C (literature[69] 177° to 178°C). The mixed melting point of the cis-4-methyl-cis, -cis-hexahydrophthalic acid and trans-4-methyl-cis, cis-hexahydrophthalic acid was 165° to 170°C.

10. Preparation of trans-4-Methyl-cis, trans-hexahydrophthalic Acid (72).[69]

A 4-gm sample of methyl cis-4-methyl-cis, cis-hexahydrophthalate and 7 gm of sodium methoxide in 200 ml of dry methanol were heated to reflux for 15 hr in a 500-ml round-bottomed flask equipped with a reflux condenser and calcium chloride drying tube. The methanol was removed by distillation, and 100 ml of cold water was added to the residue. The aqueous solution was heated to reflux for 2 hr. After cooling to room temperature, the reaction mixture was washed with 50 ml of ether and then acidified with dilute hydrochloric acid. The acidified solution was extracted with ether. After drying over magnesium sulfate, the ether was removed by distillation to yield a white solid. This solid was crystallized from aqueous methanol to yield 2 gm of trans-4-methyl-cis, trans-hexahydrophthalic acid, mp 183° to 185°C (literature[69] 181° to 182°C).

11. Preparation of 3-Carboxy-5-hydroxy-7-n-amylcoumarin (75).

A mixture of 3.7 gm (0.02 mole) of 2, 6-dihydroxy-4-n-amylbenzaldehyde, 7.4 gm (0.08 mole) of cyanoacetic acid, and 74 ml of 20% sodium hydroxide solution was stirred under a nitrogen atmosphere at room temperature for 12 hr. The reaction mixture was poured into 200 ml of cold 20% sulfuric acid and stirred at room temperature for 10 hr. The yellow solid that formed was isolated and crystallized twice from methanol to yield 4 gm (72% yield) of (75), mp 198° to 199°C.

Analysis of $C_{15}H_{16}O_5$:

Calculated: C, 65.20; H, 5.84
Found: C, 65.0; H, 5.9

IR data: $\nu C{=}O$ 1725 cm^{-1} and 1665 cm^{-1}.

12. Preparation of cis-1-Hydroxy-3-n-amyl-9-methyl-6a, 7, 10, -10a-tetrahydro-6-dibenzopyrone (76).

A mixture of 4 gm (0.014 mole) of (75), 2.3 gm of freshly distilled isoprene, 5 ml of dry xylene, and a few crystals of hydroquinone were sealed into a heavy-walled pyrex tube. The tube was placed in a steel bomb and heated with shaking to 180°C for 18 hr. The glass tube was cooled in a dry ice-acetone bath, opened, and the contents washed out with benzene. The volatile components were removed by distillation under reduced pressure. The resulting residue was dissolved in cyclohexane and placed on a chromatographic column packed with 150 gm of silica gel (Mallinckrodt). The column was developed with a cyclohexane-methanol (20:1) solvent system to give a fast-moving yellow band followed by a reddish-brown band. The slower brown band yielded 0.85 gm of a brown resin that could not be purified. The yellow band yielded 3.5 gm of a pale-yellow, viscous liquid that crystallized from hexane to yield 3.3 gm (79% yield) of (76), mp 123° to 125°C.

Analysis of $C_{19}H_{24}O_3$:

Calculated: C, 75.97; H, 8.05
Found: C, 76.1; H, 8.0

UV maxima: 283 (ε 2520), 277 mμ (ε 2250). IR data: νOH 3420 cm^{-1}; $\nu C{=}O$ 1745 cm^{-1}.

13. Preparation of 1-Hydroxy-3-n-amyl-9-methyl-6a, 7, 8, 9, 10, -10a-hexahydro-6-dibenzopyrone.

A 0.98-gm sample of (76) dissolved in 25 ml of ethyl acetate and 0.1 gm of platinum oxide catalyst were hydrogenated in a Parr apparatus for 2 hr at room temperature. The catalyst was removed by filtration and the ethyl acetate distilled from the filtrate under reduced pressure. The residue was crystallized from hexane to yield 0.65 gm of a white crystalline solid, mp 136° to 137°C.

Analysis of $C_{19}H_{26}O_3$:

Calculated: C, 75.46; H, 8.67
Found: C, 75.4; H, 9.1

IR data: $\nu C{=}O$ 1725 cm^{-1}.

14. Treatment of 1-Hydroxy-3-n-amyl-9-methyl-6a, 7, 8, 9, 10, -10a-hexahydro-6-dibenzopyrone With Ozone.

A 0.2-gm sample of 1-hydroxy-3-n-amyl-9-methyl-6a, 7, 8, 9, 10, -10a-tetrahydro-6-dibenzopyrone was dissolved in 50 ml of glacial acetic acid, and a stream of ozonized oxygen was bubbled slowly through the solution for 4 hr. A 4% solution of hydrogen peroxide (25 ml) was added to the reaction mixture. After standing for 12 hr at room temperature, the solvent was removed by distillation under reduced pressure to yield 4 mg of a white crystalline solid, mp 172° to 174°C. The IR spectrum of this solid was identical to the IR spectrum of cis-4-methyl-cis, cis-hexahydrophthalic acid (70).

15. Preparation of 3-Acetyl-5-hydroxy-7-n-amylcoumarin (78).

A solution of 1.88 gm (0.009 mole) of 2, 6-dihydroxy-4-n-amylbenzaldehyde and 1.3 gm (0.01 mole) of ethyl acetoacetate dissolved in 5 ml of methanol was cooled to 0°C in an ice bath. A solution of 6 drops of piperidine in 2 ml of methanol was added with stirring. The reaction mixture was removed from the ice bath after 1 hr and allowed to stand at room temperature for 24 hr. After adding 3 ml of dilute hydrochloric acid, the reaction mixture was cooled in an ice bath. The red solid that formed was isolated and crystallized twice from benzene to yield 0.8 gm (31% yield) of pale-yellow needles, mp 195° to 197°C. IR data: νOH 3200 cm^{-1}, νC=O 1750 and 1660 cm^{-1}.

16. Preparation of 1-Hydroxy-3-n-amyl-6a-acetyl-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone (79).

A mixture of 0.42 gm (0.002 mole) of (78), 1 ml of freshly distilled isoprene, and 2 ml of dry xylene was sealed in a pyrex tube under nitrogen atmosphere. The tube was placed in a steel bomb and heated to 180°C with shaking for 16 hr. The tube was cooled in a dry ice-acetone bath, opened, and the contents washed out with benzene. The solvent and excess isoprene were distilled off under reduced pressure. The residue was crystallized from hexane-ether (10:1) to yield 0.42 gm (80% yield) of pale-yellow needles, mp 173° to 175°C.

Analysis of $C_{21}H_{26}O_4$:

Calculated: C, 73.66; H, 7.65
Found: C, 73.3; H, 7.9

UV maxima: 285 (ε 2820), 280 mμ (ε 2570). IR data: νC=O 1750 and 1705 cm^{-1}.

17. Hydrolysis of (79).

A solution of 0.1 gm of (79) and 0.1 gm of potassium hydroxide in 10 ml of methanol was allowed to stand at room temperature for 2 days. The reaction mixture was poured into 75 ml of 5% hydrochloric acid and the brown oil that resulted was extracted with benzene. The benzene solution was placed on a column packed with 50 gm of silica gel. The column was eluted with benzene-ether (25:1) to yield a colorless resin. This resin was crystallized from hexane to yield a white solid, mp 120° to 122°C. The IR spectrum of this solid was identical to the IR spectrum of (76).

A solution of 0.1 gm of (79) dissolved in 5 ml of methanol and 20 ml of 5% sodium hydroxide solution was refluxed for 2 hr. Acidification of the hydrolysis mixture yielded an oil. This oil was extracted with ether. The ether was distilled off and the residue crystallized from hexane to yield (76).

18. Preparation of 3-Acetyl-5-hydroxy-7-methylcoumarin (84).

A mixture of 1 gm (0.006 mole) of 2,6-dihydroxy-4-methylbenzaldehyde and 0.86 gm (0.006 mole) of ethyl acetoacetate was placed in a stoppered 10-ml Erlenmeyer flask and cooled in an ice bath. A solution of 3 drops of piperidine in 5 ml of absolute methanol was added slowly. The reaction flask was removed from the ice bath and allowed to stand at room temperature for 24 hr. During this time the reaction mixture turned from a pale yellow to a dark red. The reaction mixture was cooled in an ice bath and the red solid that formed was isolated, dissolved in heptane, and treated with 0.1 gm of activated charcoal. After filtering and cooling in an ice bath, the heptane solution yielded 0.4 gm (30% yield) of a pale-yellow solid, mp 273° to 275°C.

Analysis of $C_{12}H_{10}O_4$:

Calculated: C, 66.05; H, 4.62
Found: C, 65.6; H, 4.2

19. Preparation of 1-Hydroxy-3,9-dimethyl-6a-acetyl-6a,7,10,-10a-tetrahydro-6-dibenzopyrone (85).

A mixture of 1.4 gm (0.006 mole) of (84), 4 ml of isoprene, 10 ml of dry xylene, and 0.1 gm of hydroquinone was placed in a heavy-walled pyrex tube. The tube was cooled in a dry ice-acetone bath and sealed off under a nitrogen atmosphere. After the tube had warmed to room temperature, it was placed in a steel bomb and heated with shaking to 180°C for 36 hr. The

pyrex tube was cooled in a dry ice-acetone bath, opened, and the contents washed out with ether. The solvent was removed by distillation under reduced pressure. The solid residue was crystallized twice from benzene to yield 0.84 gm (47% yield) of white needles, mp 213° to 215°C.

Analysis of $C_{17}H_{18}O_4$:

Calculated: C, 71.31; H, 6.34; O, 22.35
Found: C, 71.1; H, 6.8; O, 22.0

UV maxima: 284 (ε 2390); 278 mμ (ε 2350). IR data: $\nu C{=}O$ 1755 and 1700 cm^{-1}.

20. Preparation of cis-1-Hydroxy-3,9-dimethyl-6a,7,10,10a-tetrahydro-6-dibenzopyrone (87).

A mixture of 0.2 gm (0.007 mole) of (85), 0.1 gm of potassium hydroxide, and 10 ml of methanol was allowed to stand at room temperature for 48 hr. The reaction mixture was poured into 100 ml of cold 5% hydrochloric acid. The solid that formed was isolated and crystallized twice from petroleum ether-ether (9:1) to yield 0.1 gm (58% yield) of a white solid, mp 232° to 234°C.

Analysis of $C_{15}H_{16}O_3$:

Calculated: C, 73.75; H, 6.60
Found: C, 73.5; H, 7.1

A mixture of 0.2 gm of (85) and 0.2 gm of potassium hydroxide in 10 ml of water and 10 ml of methanol was refluxed for 2 hr. The reaction mixture was poured into 100 ml of cold 5% hydrochloric acid. The solid that formed was isolated and crystallized from petroleum ether-ether (9:1) to yield a compound melting at 232° to 234°C. The IR spectrum of this compound was identical to the material obtained by the mild hydrolysis of (85) at room temperature.

21. Condensation of Isoprene with 3-Carboxy-5-acetoxy-7-methylcoumarin (88).

A mixture of 3 gm (0.01 mole) of 3-carboxy-5-hydroxy-7-methylcoumarin (86), 50 ml of isopropenyl acetate, and 0.1 gm of p-toluenesulfonic acid was refluxed for 2 hr in a 100-ml round-bottomed flask equipped with a reflux condenser and protected from the atmosphere by a calcium sulfate drying tube. The cooled reaction mixture was diluted with 200 ml of ether.

The ether solution was washed with 50 ml of 5% sodium bicarbonate solution and then with water. After drying over magnesium sulfate, the ether and excess isopropenyl acetate were distilled off under reduced pressure. The resulting crude (88) was crystallized from benzene to yield 3 gm (90% yield) of (88), pale-yellow solid, mp 144° to 146°C.

A 2.5-gm sample of the above solid dissolved in 7 ml of dry xylene was treated with 4 ml of isoprene in the manner described for the preparation of (85) to yield 1.4 gm of a colorless glass that could not be crystallized. This material was identified as cis-1-acetoxy-3,9-dimethyl-6a,7,10,10a-tetrahydro-6-dibenzopyrone (89) from its NMR spectrum:

CH_3: 1.60 ppm (singlet), 2.22 ppm (singlet), and 2.28 ppm (singlet)

CH_2: 1.95 and 2.63 ppm

CH (10a): ca 3.0 ppm (broad)

CH olefinic: 5.36 ppm (broad singlet)

CH phenyl: 6.75 and 6.70 ppm

22. Condensation of Isoprene With 3-Carboxy-5-hydroxy-7-methylcoumarin (86).

A mixture of 2 gm of (86), 4 ml of isoprene, and 7 ml of dry xylene was heated at 205°C in a sealed tube for 18 hr to yield 1.1 gm of a compound melting at 230° to 232°C. The IR spectrum of this compound was identical to the IR spectrum of (87).

23. Acetylation of cis-1-Hydroxy-3,9-dimethyl-6a,7,10,10a-tetrahydro-6-dibenzopyrone (87).

A mixture of 1.3 gm of (87), 25 ml of isopropenyl acetate, and 0.1 gm of p-toluenesulfonic acid was refluxed in a 100-ml, round-bottomed flask equipped with a reflux condenser and drying tube for 3 hr. The cooled reaction mixture was diluted with 100 ml of ether, washed with 50 ml of 5% sodium bicarbonate solution, and then washed with 50 ml of water. After drying over magnesium sulfate, the ether and excess isopropenyl acetate were removed by distillation under reduced pressure to yield 1.3 gm of a colorless glass. The IR spectrum of the resulting (89) was identical to the IR spectrum of the compound obtained from the addition of isoprene to 3-carboxy-5-acetoxy-7-methylcoumarin (88).

24. Attempted Isomerization of cis-1-Acetoxy-3,9-dimethyl-6a,7,10,10a-tetrahydro-6-dibenzopyrone (89).

Sodium hydride (0.3 gm) was added to 25 ml of dry dimethylsulfoxide (distilled from calcium hydride).[70] The resulting mixture was heated to 65° to 70°C for 1 hr under a nitrogen atmosphere. A solution of 1.3 gm of (89) in 15 ml of dry dimethyl sulfoxide was added to the solution of sodium methylsulfinyl carbanion. The red reaction mixture was heated to 50°C for 4 hr. The cooled reaction mixture was poured into 200 ml of cold 5% hydrochloric acid. The aqueous solution was extracted with 100 ml of ether. After drying over magnesium sulfate, the ether was distilled off to yield a black resin. This resin was chromatographed on a column packed with 100 gm of silica gel. The column was eluted with 5% ether in benzene to yield 0.13 gm of cis-1-hydroxy-3,9-dimethyl-6a,7,10,10a-tetrahydro-6-dibenzopyrone (87).

A 0.58-gm sample of (89) was refluxed with 2 gm of sodium methoxide in 50 ml of dry xylene for 20 hr. The cooled reaction mixture was poured into 100 ml of cold water. The aqueous solution was acidified with 5% sulfuric acid. The xylene and water layers were separated, and the water layer was washed with 50 ml of ether. After the combined ether and xylene phases were dried over magnesium sulfate, the solvents were removed by distillation under reduced pressure to yield 0.4 gm of cis-1-hydroxy-3,9-dimethyl-6,7,10,10a-tetrahydro-6-dibenzopyrone (87).

25. Attempted Isomerization of cis-1-Hydroxy-3,9-dimethyl-6a,7,10,10a-tetrahydro-6-dibenzopyrone (87).

A 0.51 gm sample of (87) was heated with 20 gm of ammonium acetate at 215°C for 1 hr. The cooled reaction mixture was poured into 50 ml of cold water. The solid that formed was extracted with ether. After drying over magnesium sulfate, the ether was removed by distillation to yield 0.3 gm of starting material (87).

26. Preparation of 2,6-Dihydroxy-3-carbethoxy-4-n-amylbenzaldehyde.[71]

A solution of 5 gm (0.02 mole) of ethyl 2,4-dihydroxy-6-n-amylbenzoate in 75 ml of dry ether was placed in a 500-ml, three-necked, round-bottomed flask equipped with a mercury seal stirrer, a reflux condenser, and a thermometer. The solution was cooled to -5°C in an ice-salt bath, and 3.5 gm (0.02 mole) of zinc cyanide was added. A solution of 5.3 gm (0.04 mole) of aluminium chloride in 75 ml of dry ether was then added. Dry hydrogen chloride was bubbled through the reaction mixture with cooling for 5 hr. After the careful addition of 75 ml of water, the mixture was heated

to 100°C for 30 min. A yellow precipitate formed that was isolated and crystallized from aqueous methanol. Recrystallization from ethanol and water yielded 4 gm (72% yield) of a pale-yellow, crystalline product melting at 35° to 37°C. A good elemental analysis for the desired aldehyde could not be obtained from this solid. The 2,4-dinitrophenylhydrazone of the product was readily purified to give the correct analysis for the desired product.

The 2,4-dinitrophenylhydrazone prepared by a standard method[72] was purified by crystallization first from ethyl acetate and then from acetic acid. The bright-yellow 2,4-dinitrophenylhydrazone melted with decomposition at 242° to 244°C.

Analysis of $C_{21}H_{24}N_4O_8$:

Calculated: C, 54.78; H, 5.25; N, 12.17
Found: C, 55.01; H, 5.4; N, 12.3

27. Preparation of 3-Carboxy-5-hydroxy-6-carbethoxy-7-n-amylcoumarin (90).

To a mixture of 4 gm (0.014 mole) of crude 2,6-dihydroxy-3-carbethoxy-4-n-amylbenzaldehyde and 2 gm (0.024 mole) of cyanoacetic acid was added 100 ml of cold 20% sodium hydroxide solution. The resulting dark solution was stirred for 3 hr. After standing at room temperature for 24 hr, the reaction mixture was acidified with dilute hydrochloric acid. The resulting yellow solid was refluxed with 4% hydrochloric acid for 1 hr. A pale-yellow solid was isolated and crystallized from methanol. Recrystallization from isopropanol yielded 4 gm (94% yield) of (90), a pale-yellow solid, mp 151° to 153°C.

Analysis of $C_{18}H_{20}O_7$:

Calculated: C, 62.06; H, 5.79
Found: C, 61.7; H, 5.6

28. Preparation of 1-Hydroxy-2-carbethoxy-3-n-amyl-9-methyl-6a,7,10,10a-tetrahydro-6-dibenzopyrone (91).

A mixture of 3 gm (0.008 mole) of (90), 1.1 gm of freshly distilled isoprene, 10 ml of dry xylene, and a few crystals of hydroquinone was sealed into a heavy-walled pyrex tube under a nitrogen atmosphere. The tube was placed in a steel bomb and heated with shaking to 180°C for 18 hr. After the pyrex tube was cooled in a dry ice-acetone bath, it was opened and the contents

washed out with benzene. The volatile components were distilled off under reduced pressure. The resulting residue was dissolved in hexane and placed on a chromatographic column packed with 150 gm of silica gel. The column was eluted with a hexane-ether (19:1) solvent system. The major fraction that appeared as a yellow band on the column yielded 2.4 gm of a pale-yellow, viscous liquid. This liquid was dissolved in a minimum amount of methanol. After the methanol solution was cooled in an ice bath for several hours, 2 gm (63% yield) of a white crystalline product melting at 70° to 72°C was isolated.

Analysis of $C_{22}H_{28}O_5$:

Calculated: C, 70.94; H, 7.58
Found: C, 70.8; H, 7.6

UV maxima: 304 (ε 5530), 260 (ε 12500), 218 mμ (ε 34200).
IR data: $\nu C{=}O$ 1775, 1770, and 1660 cm^{-1}.

29. Preparation of 5-(2,6-Dihydroxy-4-n-amylphenyl)-4-(1-methyl-1-hydroxyethyl)-1-methylcyclohexene (80).

In a dry, 500-ml, round-bottomed flask equipped with a reflux condenser, dropping funnel, and magnetic stirring bar, and under a nitrogen atmosphere, was placed 2.4 gm (0.1 mole) magnesium turnings and 25 ml of dry ether. Approximately 15 ml of a solution of 14.5 gm (0.1 mole) of methyl iodide in 75 ml of dry ether was added through the dropping funnel. The flask was warmed slightly to start the reaction. After the ether had begun to reflux, the methyl iodide solution was added at a rate to maintain reflux. After the addition was completed, the reaction mixture was refluxed for 30 min. A solution of 3 gm (0.01 mole) of (76) in 150 ml of dry benzene was added to the Grignard reagent through the dropping funnel, and the reaction mixture refluxed for 12 hr. The reaction mixture was cooled in an ice bath and 150 ml of cold 10% ammonium chloride solution added slowly. The aqueous layer was washed with 50 ml of benzene. The organic layer was combined with the benzene washings, washed with water, and dried over magnesium sulfate. The solvent was distilled from the dried ether-benzene solution under reduced pressure. The dark-red residue was dissolved in 50 ml of benzene and placed on a chromatographic column packed with 350 gm of silica gel. The column was eluted with benzene-ether (9:1) to yield a yellow resin. This resin was dissolved in 50 ml of hexane and allowed to stand at room temperature for 2 days. The hexane solution deposited 1.67 gm (50% yield) of (80), white crystalline solid, mp 118° to 119°C.

Analysis of $C_{21}H_{32}O_3$:

Calculated: C, 75.86; H, 9.70
Found: C, 75.6; H, 9.6

30. Preparation of cis-1-Hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyran (81).

A solution of 2 gm of (80) and 10 ml of concentrated sulfuric acid in 100 ml of methanol was stirred at room temperature for 24 hr. The reaction mixture was neutralized with solid sodium bicarbonate. After the inorganic salts were removed by filtration, the methanol was distilled off at room temperature under reduced pressure. The residue, which still contained inorganic salts, was dissolved in water and extracted with 50 ml of benzene. After drying over magnesium sulfate, the benzene solution was placed on a chromatographic column packed with 150 gm of silica gel. The column was eluted with benzene that was collected in 20-ml cuts. The cuts were analyzed by thin-layer chromatography (TLC) on silica gel, and combined into two fractions. Fraction 1 yielded 0.83 gm of a red resin after removal of the benzene by distillation under reduced pressure. Fraction 2 yielded 0.45 gm of a white solid. This solid was crystallized twice from hexane to yield a white crystalline solid, mp 70° to 72°C.

Analysis of $C_{22}H_{34}O_3$:

Calculated: C, 76.26; H, 9.89
Found: C, 76.1; H, 9.9

UV maxima: 210 (ε 47000), 275 (ε 4030), 283 (ε 4380), and 319 mμ (ε 1450). This product appeared to be a methoxy derivative of (81).

The resinous fraction 1 was rechromatographed on 220 gm of silica gel-silver nitrate (5:1) using benzene as the eluant. Cuts of 20 ml were taken. Cuts 1 to 5 yielded 0.006 gm of a colorless resin that crystallized from a petroleum ether solution to yield 3 mg of a white solid, mp 108° to 109°C. This solid was not identified. Cuts 6 to 9 yielded 0.77 gm of a colorless resin that rapidly discolored when exposed to the air. A solution of this resin in 10 ml of petroleum ether (30° to 60°C) yielded 0.38 gm of cis-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyran (81), white crystals in the form of rosettes, mp 64° to 65°C, after standing at -5°C for 4 wk.

Analysis of $C_{21}H_{30}O_2$:

Calculated: C, 80.21; H, 9.62
Found: C, 80.4; H, 9.7

UV maxima: 283 (ε 1210), 275 (ε 1170), 212 mμ (ε 39300).

Cut 10 from the silica gel-silver nitrate column yielded 0.03 gm of a colorless resin. A petroleum ether solution of this resin deposited 3 mg of a white solid, mp 50° to 53°C.

UV maxima: 283 (ε 1150), 278 (ε 1130), 229 (ε 9200), and 210 mμ (ε 39,200). This compound was not completely identified, but it appeared to be a double-bond isomer of (81).

31. Dehydrogenation of (81).[44]

A 0.2-gm sample of (81) and 0.4 gm of sulfur were mixed in a test tube and heated to 160° to 170°C for 10 hr. The cooled reaction mixture was extracted with boiling hexane. The hexane extract was placed on a chromatographic column packed with 100 gm of silica gel. The column was eluted with benzene to yield a red resin. This resin was treated with 10 ml of acetic anhydride and 1 gm of sodium acetate at room temperature for 5 days. The reaction mixture was poured into 50 gm of ice, and the resulting oil was extracted with 100 ml of petroleum ether (30° to 60°C). The petroleum ether solution was washed with 50 ml of 5% sodium bicarbonate solution and then with water. After drying over magnesium sulfate, the petroleum ether was removed by distillation. The residue was crystallized from 2 ml of petroleum ether (30° to 60°C) to yield 0.1 gm of colorless prisms, mp 75° to 76°C. A mixture of the above solid and an authentic sample of cannabinol acetate showed no depression in the melting point. The IR spectrum of the above solid was identical to the IR spectrum of cannabinol acetate.

32. Preparation of 1-Hydroxy-3-n-amyl-6, 6, 9-trimethyl-7, 8, 9, 10-tetrahydro-6-dibenzopyran (44).[48]

A solution of 5 gm (0.028 mole) of 3, 5-dihydroxyamylbenzene, 10.25 gm (0.056 mole) of ethyl 5-methylcyclohexanone-2-carboxylate, and 3 ml of phosphorus oxychloride in 30 ml of dry benzene was refluxed for 5 hr in a 100-ml round-bottomed flask equipped with a magnetic stirrer, reflux condenser, and calcium chloride drying tube. The cooled reaction mixture was diluted with 50 ml of ether and washed with three 50-ml portions of water. After drying over magnesium sulfate, the solvents were distilled

benzene and extracted with four 50-ml portions of a solution containing 4 gm of potassium hydroxide and 6 gm of sodium sulfite in 200-ml of water. The benzene solution was washed with water and dried over sodium sulfate.

The basic extract was acidified with 2% sulfuric acid and extracted with 200 ml of ether. The ether solution was reextracted with 100 ml of 5% sodium carbonate solution and then dried over sodium sulfate. Evaporation of the ether solution under reduced pressure yielded 20 mg of a red resin that appeared to contain cannabidiolic acid. The acidified sodium carbonate solution yielded a few milligrams of an unidentified acidic substance.

The benzene solution containing the bulk of the material was placed on a chromatographic column (1.5 by 60 in.) packed with 600 gm of silica gel. The column was eluted with benzene to yield 3.8 gm of a phenolic fraction composed of primarily tetrahydrocannabinol and small amounts of cannabinol and cannabidiol.

A similar extraction of a 108-gm sample of marijuana yielded 1.0 gm of a tetrahydrocannabinol fraction by chromatography on alumina and silica gel. A petroleum ether solution of this fraction yielded 10 mg of a white solid, mp 86° to 88°C. This solid was shown to be a mixture of three tetrahydrocannabinols by TLC on silica gel-silver nitrate (5:1), using benzene as the developer.

The phenolic fractions from the two extractions were combined and rechromatographed with 300 gm of silica gel as the eluting solvent. The phenolic material moved down the column as a broad yellow band. This band was taken from the column in 20-ml cuts. The cuts were analyzed by paper chromatography on 20- by 20-cm sheets of Whatman No. 3 MM filter paper saturated with dimethylformamide and developed with cyclohexane saturated with dimethylformamide. The paper chromatograms showed that the material recovered (3 gm) from the column was composed of two tetrahydrocannabinols contaminated with a small amount of cannabinol. This material was rechromatographed on a column (1.5 by 60 in.) packed with 500 gm of silica gel. The column was eluted with benzene and cuts of 20 ml were taken and analyzed by paper chromatography on Whatman No. 3 MM filter paper. The cuts from the column yielded 1.5 gm of a mixture of two tetrahydrocannabinols. The cuts that followed yielded 1.0 gm of (42).

The fraction, which consisted of a mixture of tetrahydrocannabinols, was separated by column chromatography on 400 gm of silica gel-silver nitrate (5:1) using benzene as the eluting solvent. Cuts of 25 ml were taken and analyzed by TLC on silica gel-silver nitrate (5:1) using benzene as

the developer. Identical cuts were combined, washed with water, and dried over magnesium sulfate, and the solvent was distilled off under reduced pressure to yield 0.73 gm of the tetrahydrocannabinol (42), 0.11 gm of the tetrahydrocannabinol (95), and 2 mg of a resin that crystallized from petroleum ether to yield a solid, mp 143° to 145°C. Tetrahydrocannabinol (42), $[\alpha]_D^{27}$ -167° (c 0.353, absolute ethanol).

UV maxima: 209 (ε 37,600), 277 (ε 1330), and 284 mμ (ε 1370). Tetrahydrocannabinol (93), $[\alpha]_D^{29}$ -260° (0.700, absolute ethanol). UV maxima: 209 (ε 41,000), 276 (ε 1330), 283 mμ (ε 1390).

Analysis of $C_{21}H_{30}O_2$:

Calculated: C, 80.21; H, 9.6
Found: (2) C, 79.9; H, 9.8

Solid mp 143° to 145°C, UV maxima: 299 (ε 480), 284 (ε 1750), 276 (ε 1680), 230 (ε 17,300), and 212 mμ (ε 41,600).

34. Isolation of Tetrahydrocannabinol From Mexican Marijuana.

A 120-gm sample of Mexican marijuana was extracted with 1,500 ml of petroleum ether and the phenolic fraction isolated in the manner described above. TLC on silica gel-silver nitrate (5:1) showed the phenolic fraction contained cannabidiol, cannabinol, and two tetrahydrocannabinols. The tetrahydrocannabinols were isolated by column chromatography on silica gel-silver nitrate (5:1) using benzene as the eluting solvent. A yield of 0.43 gm of (42) and 0.06 gm of (93) was isolated. These materials were identified by their IR spectra.

35. Isolation of Tetrahydrocannabinol From Spanish Marijuana.

A 380-gm sample of Spanish marijuana grown in 1962 was extracted with 4 ℓ of petroleum ether (30° to 60°C) at 20°C with the exclusion of light. The petroleum ether was decanted from the solid material, which was then washed with an additional liter of petroleum ether. The combined extract and wash solution were filtered, and the petroleum ether was distilled off under reduced pressure to yield 20 gm of a black resin. This resin was shaken with 200 ml of methanol until all of the material had dissolved. The methanol solution was cooled to -5°C overnight, and the green waxy solid that precipitated was removed by filtration. The methanol was distilled from the filtrate under reduced pressure to yield 14 gm of a black resin. This resin was dissolved in 280 ml of hexane and placed on a chromatographic column packed with 450 gm

of silica gel. The column was eluted with benzene to yield 3.0 gm of a phenolic fraction. The phenolic fraction was rechromatographed with benzene on a column packed with 500 gm of silica gel-silver nitrate (5:1). Cuts of 20 ml were taken and analyzed by TLC on silica gel-silver nitrate (5:1), using benzene as the developing solvent. Like cuts were combined, washed with water, and dried over magnesium sulfate, and the benzene was removed by distillation under reduced pressure to yield 0.6 gm of cannabidiol, 0.5 gm of cannabinol, and 0.8 gm of the tetrahydrocannabinol (42), $[\alpha]_D^{27}$ -166° (c 0.600, absolute ethanol).

36. A Second Method for Isolation of Tetrahydrocannabinol From Marijuana.

A 22.5-kg sample of 3-yr-old Mexican marijuana obtained from the US Treasury Department was extracted by slowly percolating 20 gal of methanol through the marijuana leaves. The methanol was removed from the dark-green extract by distillation under reduced pressure. The resulting black residue was extracted at room temperature with 4 *l* of petroleum ether (30° to 60°C). The petroleum ether was removed by distillation under pressure to yield 242 gm of a black resin. A phenolic fraction was obtained from this resin by chromatographing twice on Florisil and then twice on alumina (neutral, activity grade III) using benzene as the eluting solvent. The phenolic fraction was dissolved in 50 ml of pyridine and added to a solution of 25 ml of acetic anhydride in 100 ml of pyridine. The resulting mixture was heated to 50°C for 12 hr. After the reaction mixture had cooled to room temperature, it was poured into 500 ml of ice water. The resin that separated was dissolved in 200 ml of petroleum ether (30° to 60°C). The petroleum ether solution was washed with water, 5% sodium bicarbonate solution, water, 5% sulfuric acid solution, and finally with water. After the solution was dried over anhydrous magnesium sulfate, the petroleum ether was removed by distillation under reduced pressure. The resulting residue was dissolved in 50 ml of pentane and cooled to 0°C. The cold solution was seeded with a crystal of cannabinol acetate and then allowed to stand at 0°C for 24 hr. The 11 gm of cannabinol acetate (mp 75° to 76°C) that crystallized out was isolated. The noncrystalline residue from the pentane solution was dissolved in cyclohexane and placed on a column packed with 300 gm of silicic acid that had been treated with 60 gm of silver nitrate. The column was eluted with 5% ether in cyclohexane to yield three fractions. The fastest-running fraction on the column yielded a pale-yellow resin. This resin crystallized from methanol to yield a white crystalline solid, mp 190° to 193°C. The IR and NMR spectra of this solid showed this material to be an acetate ester; however, the material, is not aromatic and does not correspond to any of the compounds of known structure present in marijuana. The second fraction removed from the column yielded

4.9 gm of a resin. This resin was composed mainly of the tetrahydrocannabinol acetate (42a). The third fraction from the column yielded 3 gm of crystalline cannabinol acetate. Final purification of the tetrahydrocannabinol acetate was accomplished by chromatography on silicic acid using benzene as the eluting solvent. The yield of tetrahydrocannabinol acetate (42a) was 4.5 gm. The purity of this material was shown by TLC. Thin-layer chromatograms of (42a) on silica gel-silver nitrate, silica gel, and neutral alumina showed only one component. Benzene was used as the developing solvent for the thin-layer chromatograms. The structure of (42a) was demonstrated by conversion of (42a) to the tetrahydrocannabinol (42) of known structure.

37. Conversion of Tetrahydrocannabinol Acetate (42a) to trans-1-Hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 8, 10a-tetrahydro-6-dibenzopyran (42).

A solution of 1.2 gm of (42a) in 100 ml of dry ether was added over 15 min to a rapidly stirred solution of 0.2 gm of lithium aluminum hydride in 200 ml of dry ether cooled to 0°C. The reaction mixture was stirred at 0°C for 2 hr, after completion of the addition. Ethyl acetate (5 ml) was added cautiously to the reaction mixture. After the reaction mixture was stirred for 15 min, 10 ml of acetic acid and then 150 ml of water were added. The reaction mixture was placed in a separatory funnel and 200 ml of pentane was added. The aqueous layer was separated and discarded. The organic layer was washed with 100 ml of 5% sodium bicarbonate solution and then with 300 ml of water in three portions. After the organic layer was dried over magnesium sulfate, the solvent was removed by distillation under reduced pressure to yield 1.04 gm (98% yield) of a colorless resin. This resin had IR and NMR spectra that were identical to the natural tetrahydrocannabinol (42).

38. Hydrogenation of trans-1-Hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 8, 10a-tetrahydro-6-dibenzopyran (42).

An 0.2-gm sample of (42) dissolved in 25 ml of ethyl acetate and 0.05 gm of platinum oxide were hydrogenated in a Parr apparatus at 45 psi and room temperature for 1 hr. After removal of the catalyst by filtration, the ethyl acetate was distilled off under reduced pressure. The reaction product, a bright-yellow resin, was purified by chromatography on 40 gm of silica gel using benzene as the eluting solvent. A yield of 0.15 gm of a colorless resin was obtained from the column. The purity of this material was demonstrated by the fact that TLC on silica gel (developers: benzene and 5% methanol in hexane) and silica gel-silver nitrate (developers: benzene and 2% ethyl acetate in benzene) showed the presence of only one component, $[\alpha]_D^{27}$ -108° (c 0.507, absolute ethanol).

Analysis of $C_{21}H_{32}O_2$:

Calculated: C, 79.68; H, 10.19
Found: C, 79.5; H, 10.5

39. Hydrogenation of trans-1-Hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyran (93).

A 0.08-gm sample of (93) and 0.02 gm of platinum oxide in 15 ml of ethyl acetate were hydrogenated in a Parr apparatus at 45 psi and at room temperature for 1 hr. The catalyst was removed by filtration and the ethyl acetate distilled from the filtrate under reduced pressure to yield a yellow resin. This resin was chromatographed on 50 gm of silica gel using benzene as the eluting solvent to yield 0.05 gm of a colorless resin, $[\alpha]_D^{27}$ -109° (c 0.502, absolute ethanol). The IR spectrum of this resin was identical to the IR spectrum of the compound obtained from the hydrogenation of (42).

40. Preparation of the 3, 5-Dinitrophenylurethane of trans-1-Hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyran (93).

A 0.5-gm sample of (93), 0.5 gm of 3, 5-dinitrophenylazide,[73] and 100 ml of dry petroleum ether (bp 75° to 90°C) were placed in a dry 250-ml round-bottomed flask equipped with a reflux condenser and a magnetic stirrer and protected from the air by a calcium chloride drying tube. The reaction mixture was refluxed for 6 hr. A dark-yellow solid that formed during the course of the reaction was removed by filtration. The reaction mixture was placed on a column packed with 75 gm of silicic acid (Mallinckrodt). The column was eluted with 5% ether in petroleum ether to yield a pale-yellow resin. This resin crystallized from petroleum ether (75° to 90°C) to yield a pale-yellow solid, mp 87° to 88°C.

Analysis of $C_{28}H_{33}N_3O_7$:

Calculated: C, 64.2; H, 6.35
Found: C, 63.7; H, 6.3

A 20-mg sample of the above urethane was dissolved along with 50 mg of sodium methoxide in 20 ml of methanol. The solution, which turned bright yellow, was allowed to stand at room temperature for 3 hr. The solvent was distilled under reduced pressure and the resulting residue treated with 25 ml of cold water. The water solution was extracted with 25 ml of petroleum

ether (30° to 60°C). After drying over magnesium sulfate, the petroleum was distilled off to yield a viscous residue. The IR spectrum of this residue was identical to the IR spectrum of the starting material, $\Delta^{8,9}$-tetrahydrocannabinol (93).

41. Isolation of Cannabidiolic Acid as the Diacetate (94) From West Virginian Marijuana.

A 1,600-gm batch of freshly harvested marijuana leaves was packed into four columns of 5 by 76 cm. Petroleum ether (30° to 60°C) was percolated through the columns at 0°C with the exclusion of light. A total of 7.5 gal of a greenish-yellow extract was obtained. The extract was reduced to a volume of 1 ℓ by distillation of the petroleum ether at 25°C under reduced pressure. The resulting solution was extracted with eight 50-ml portions of a solution containing 2 gm of sodium hydroxide and 2 gm of sodium sulfite per 100 ml of water. The basic extract was acidified with 5% sulfuric acid. A greenish-yellow oil precipitated. This oil was extracted with 1 ℓ of benzene. After the benzene solution was dried over sodium sulfate, the volume of the solution was reduced to 500 ml in vacuo. To the benzene solution, 50 ml of acetic anhydride and 10 gm of sodium acetate were added. This reaction mixture was allowed to stand at room temperature in the dark for 4 days and then washed with water to remove the sodium acetate and unreacted acetic anhydride. After drying over sodium sulfate, the benzene was distilled off under reduced pressure to yield 12 gm of a red oily residue. The residue was dissolved in 200 ml of petroleum ether (30° to 60°C) and placed on a column (1.5 by 24 in.) packed with 150 gm of silica gel. The column was eluted with petroleum ether-ether (9:1). A red band was eluted first from the column. This band yielded only a minor amount of material of unknown structure. The red band was followed by a blue-white fluorescent band. This band was taken off in 36 cuts of 50 ml each. Each cut was slowly evaporated under a stream of nitrogen until crystallization began. After each cut was cooled for several hours at 0°C, the white precipitates were isolated. All of the cuts yielded the same compound as demonstrated by melting points and mixed melting points. A yield of 7.25 gm of white needles from petroleum ether was obtained, mp 124° to 125°C. Recrystallization of this solid from hexane yielded dimorphic white needles, mp 96° to 98°C/124° to 125°C, $[\alpha]_D^{26}$ -60° (c 0.921, absolute ethanol), solid mp 124° to 125°C, $[\alpha]_D^{26}$ -61° (c 1.07, absolute ethanol).

Analysis of $C_{26}H_{34}O_6$:

Calculated: C, 70.89; H, 7.31; O, 21.79
Found: C, 70.5; H, 7.9; O, 21.3

42. Isolation of Cannabidiolic Acid (95) From West Virginian Marijuana.

Eight pounds of marijuana harvested in September, 1963, and kept in the dark at room temperature until June, 1964, was extracted with 9 gal of petroleum ether (30° to 60°C) for 7 days at 20°C. The petroleum ether solution was concentrated to 1 ℓ by distillation at 25°C under reduced pressure, and the concentrate was extracted with 1 ℓ of water containing 20 gm of sodium hydroxide and 20 gm of sodium sulfite. The basic extract was acidified with 5% sulfuric acid and the precipitated oil taken into 300 ml of ether. The ether solution was washed with 100 ml of 5% sodium bicarbonate and then with water. After drying over sodium sulfate, the ether was distilled off to yield 14 gm of a tan solid. A 2-gm sample of this solid was crystallized from hexane to yield a nearly white solid that melted between 120° and 130°C with decomposition and evolution of gas. The IR spectrum of this solid compared favorably to the IR spectrum of cannabidiolic acid published by Schultz and Haffner.[33] $[\alpha]_D^{26}$ -121° (c 0.941, absolute ethanol). UV maxima: 224 (ε 31,000), 260 (ε 8940), 302 mμ (ε 3900).

An ethanol solution of a sample of the above solid was allowed to stand at room temperature for 4 wk. The decomposition of the cannabidiolic acid was followed by TLC on Woelm silica gel using 10% ethyl acetate in benzene as the developer. The initial chromatogram showed only the presence of cannabidiolic acid (R_f 0.42). After 1 day, a second spot (R_f 0.71) appeared on the thin-layer chromatograms. This spot had the same R_f value as cannabidiol. After 4 wk, all of the cannabidiolic acid had disappeared. The ethanol was removed by distillation under reduced pressure and the red residue crystallized from petroleum ether to yield a white solid, mp 66° to 68°C. No depression in the melting point was noted when this compound was mixed with an authentic sample of cannabidiol.

43. Treatment of Cannabidiolic Acid With Ethanolic Hydrogen Chloride.

A 2.63-gm sample of cannabidiolic acid was dissolved in 100 ml of 0.01 N ethanolic hydrogen chloride. The solution was refluxed for 15 hr. The ethanol was distilled off under reduced pressure to yield a dark-red residue. This residue was chromatographed on a column packed with 200 gm of silica gel. The column was eluted with benzene to yield 1.7 gm of a pale-yellow resin. This resin was separated into two fractions by chromatography on a column packed with 250 gm of silica gel-silver nitrate (5:1) using benzene as the eluting solvent. Fraction 1: 0.4 gm $[\alpha]_D^{26}$ -49° (c 0.668), absolute

ethanol). UV maxima: 208 (ε 41,600), 232 (ε 10,600), 275 (ε 1360), 282 mμ (ε 1380). Fraction 1 was shown to be cis-1-hydroxy-3-n-amyl-6,6,9-trimethyl-6a, 7, 8, 10a-tetrahydro-6-dibenzopyran (96) by its NMR spectrum.

Fraction 2 (0.70 gm) $[\alpha]_D^{26}$ -279° (c 0.662, absolute ethanol) was shown to be a mixture of cis-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone (81) and trans-1-hydroxy-3-amyl-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyran (93) by its NMR spectrum.

Sulfur dehydrogenation of 50-mg samples of fractions 1 and 2 by the method described for the dehydrogenation of (81) yielded cannabinol.

44. Treatment of Cannabidiolic Acid With Methanolic Hydrochloric Acid.

A 2-gm sample of cannabidiolic acid was dissolved in 100 ml of methanol containing 3 ml of concentrated hydrochloric acid. After the solution stood at room temperature for 5 days, TLC showed that all of the cannabidiolic acid had reacted. The methanol was distilled off under reduced pressure to yield a red resin. This resin was chromatographed on a column packed with 200 gm of silica gel-silver nitrate (5:1) using benzene as the eluant to yield 0.02 gm of (96) and 0.02 gm of (93). A third product (0.75 gm) was also isolated from the column. This material, a solid, was recrystallized twice from petroleum ether (30° to 60°C) to yield colorless needles, mp 91.5° to 92.5°C, $[\alpha]_D^{26}$ -41° (c, 1.14 absolute ethanol).

Analysis of $C_{22}H_{34}O_3$:

Calculated: C, 76.26; H, 9.89; O, 13.85
Found: C, 76.2; H, 10.0; O, 13.9

UV maxima: 208 (ε 44,500), 228 (ε 11,350), 274 (ε 1190), 282 mμ (ε 1180). The structure of 1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-9-methoxy-6a, 7, 8, 9, 10, 10a-hexahydro-6-dibenzopyran (97) was assigned to this material.

A 0.38-gm sample of cannabidiolic acid dissolved in 50 ml of methanol containing 0.04 ml of concentrated hydrochloric acid was allowed to stand at room temperature for 2 days. The methanol was removed by distillation under reduced pressure to yield a red residue. This residue was dissolved in 10 ml of benzene and placed on a column packed with 150 gm of silica gel-silver nitrate (5:1). The column was eluted with benzene to yield

0.08 gm of a colorless resin, $[\alpha]_D^{26}$ -160° (c, 0.594, absolute ethanol). The IR spectrum of this resin was identical to the IR spectrum of the tetrahydrocannabinol (42). A small amount of the tetrahydrocannabinol (93) was also eluted from the column.

45. Treatment of Cannabidiolic Acid With Phosphoric Acid.

A 0.5-gm sample of cannabidiolic acid was dissolved in 50 ml of ethanol containing 7 ml of 85% phosphoric acid and refluxed for 30 min. The reaction mixture was poured into 400 ml of water, and the reaction product was extracted with 200 ml of petroleum ether. After drying over sodium sulfate, the petroleum ether was distilled off to yield a yellow resin. This resin was chromatographed on 100 gm of silica gel using benzene as the eluting solvent to yield 0.2 gm of a nearly colorless resin, $[\alpha]_D^{26}$ -130° (c, 0.646, absolute ethanol). The resin was shown to be a mixture of 30% cis- and 70% trans-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 8, 10a-tetrahydro-6-dibenzopyran. UV maxima: 209 (ε 43,000), 276 (ε 1330), 283 mμ (ε 1350).

46. Treatment of Cannabidiolic Acid With p-Toluenesulfonic Acid.

A 1-gm sample of cannabidiolic acid and 0.5 gm of p-toluenesulfonic acid monohydrate in 50 ml of toluene were refluxed for 5 hr. The cooled reaction mixture was diluted with 100 ml of petroleum ether and washed with 50 ml of 2% sodium bicarbonate solution and with 100 ml of water. After drying over magnesium sulfate, the toluene and petroleum ether were distilled off under reduced pressure to yield a brown resin. This resin was chromatographed on a column packed with 100 gm of silica gel using benzene as the eluting solvent. A bright-yellow resin was obtained from the benzene eluate. This resin was rechromatographed on 100 gm of silica gel using petroleum ether (30° to 60°C) -ether (9:1) as the eluant to yield a colorless resin, $[\alpha]_D^{26}$ -261° (c, 0.700, absolute ethanol).

UV maxima: 209 (ε 41,000), 276 (ε 1330), 283 mμ (ε 1390). The IR and NMR spectra of this resin were identical to those of trans-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyran (93).

47. Treatment of Cannabidiol With p-Toluenesulfonic Acid.

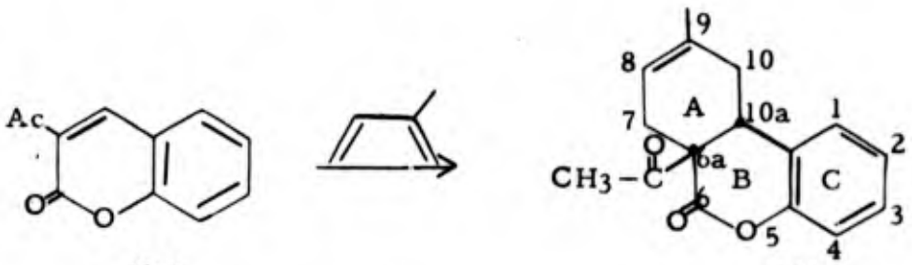
A 3.9-gm sample of cannabidiol was dissolved in 60 ml of xylene containing 0.2 gm of p-toluenesulfonic acid monohydrate. The reaction mixture was refluxed for 4 hr under a nitrogen atmosphere. After the reaction mixture had cooled, 100 ml of petroleum ether was added. The resulting solution was washed with 50 ml of 2% sodium bicarbonate solution and then

with water. After drying over magnesium sulfate, the solvent was distilled off under reduced pressure. The brown resin that resulted was chromatographed on a column packed with 250 gm of silica gel-silver nitrate (5:1) using benzene as the eluting solvent. Cuts of 15 ml were taken and analyzed by TLC on silica gel-silver nitrate. The cuts that were identical were combined, washed with water, and dried over magnesium sulfate, and the solvent was distilled off. A yield of 1.9 gm of a colorless resin was obtained, $[\alpha]_D^{26}$ -229° (c 0.530, absolute ethanol).

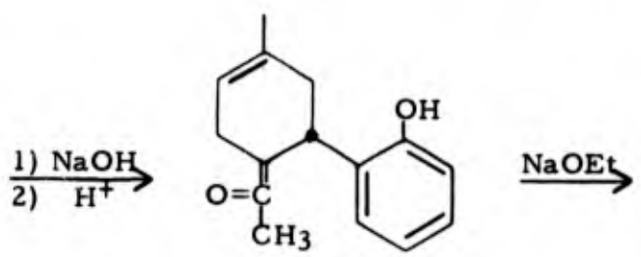
UV maxima: 208 (ε 40,600), 228 (ε 10,500), 276 (ε 1340), 283 mμ (ε 1410). The IR spectrum and NMR spectrum of this compound were identical to the IR and NMR spectra of trans-1-hydroxy-3-n-amyl-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6-dibenzopyran (93).

V. DISCUSSION.

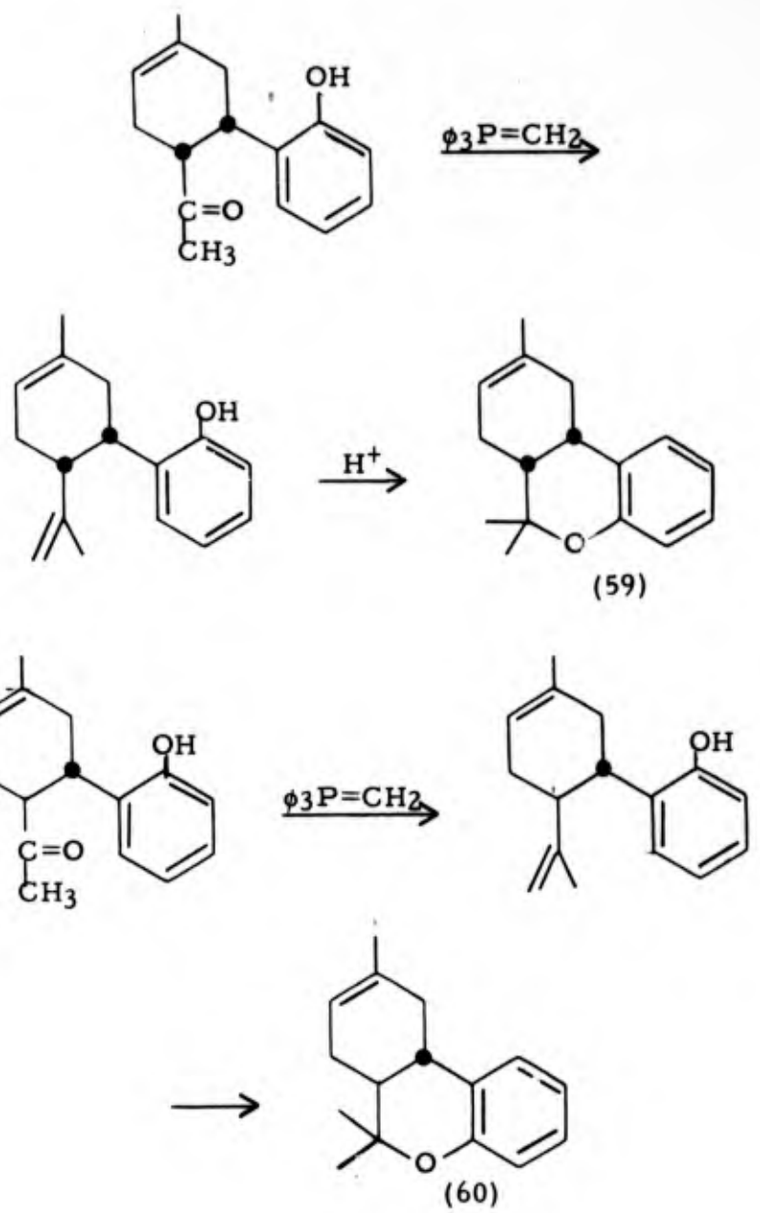
In an attempt to find a method for the synthesis of the two pairs of diastereomeric cis- and trans-1-hydroxy-3-n-amyl-6,6,9-trimethyl-6a,-7,10,10a-tetrahydro-6-dibenzopyrans by means of a Diels-Alder condensation of isoprene with an appropriately substituted coumarin, a model system, 6a-acetyl-9-methyl-6a,7,10,10a-tetrahydro-6-dibenzopyrone, was studied. The proposed route of forming the desired isomeric 6,6,9-trimethyl-6a,7,-10,10a-tetrahydro-6-dibenzopyrans (55) and (56) is illustrated by the following scheme.



(65) (66)



(67)



The dienophile, acetylcoumarin (65), was readily prepared by the condensation of salicylaldehyde and ethyl acetoacetate in the presence of piperidine. The IR spectrum of the pale-yellow crystalline compound, mp 123° to 125°C (literature[67] mp 123° to 124°C), indicated the presence of both a ketone carbonyl and a lactone carbonyl.

Isoprene was condensed with (65) to form mainly a crystalline adduct, mp 120° to 121°C. A second product, mp 106° to 108°C, accounted for about 10% of the total solid isolated but was not fully characterized. The IR spectrum of the adduct, mp 120° to 121°C, had two strong bands at 1770 and 1705 cm^{-1} in the carbonyl stretching region, which indicated the presence of two different carbonyl groups. The UV spectrum had maxima at 277 (ε 742), 268 (ε 814), and 262 mμ (ε 697) and established that the alicyclic double bond of the adduct was not conjugated with the phenyl nucleus.[65]

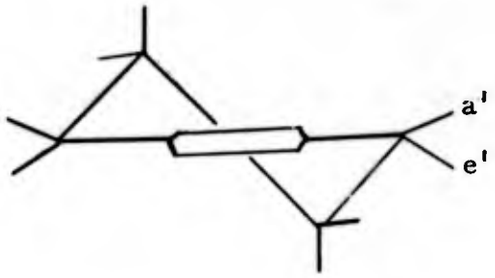
The adduct, mp 120° to 121°C, was assigned structure (66) on the basis of the NMR spectrum (table II)..

Table II. NMR Spectrum of (66) in $CDCl_3$

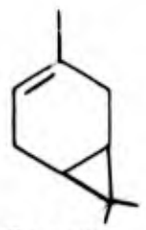
Group	Chemical shift from tetramethylsilane
	ppm
C-9 methyl	1.75 (singlet)
Acetyl methyl	2.11 (singlet)
C-10a proton	3.45 (four lines)
Olefinic proton	5.41 (broad singlet)
Aromatic protons	7.17 (multiplet)

A broad singlet for one olefinic proton and a slightly broadened singlet for three protons at 1.75 ppm established the presence of a $-\underset{H}{\underset{|}{C}}=\underset{CH_3}{\underset{|}{C}}-$ grouping in (66).[73]

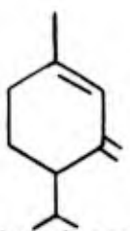
The broadness of the olefinic proton indicates that the proton is weakly coupled with the adjacent C−7 proton. Examination of Drieding models showed that in the chair form of the ring the dihedral angles between each of the C−7 protons and the olefinic C−8 proton were approximately 55°. From a plot of the theoretical coupling constant versus the dihedral angle by J. B. Strothers,[73] the coupling constant for a dihedral angle of 55° should be about 3 cps. The C−7 methylene protons of (66), however, are not equivalent and have a pseudoaxial and pseudoequatorial conformation as represented by a' and e', respectively, in cyclohexene,[74] as follows.



If no other protons are coupled with the olefinic proton, the C−7 and C−8 protons in (66) would form an ABX system of interacting nuclei, where the C−7 protons would represent the AB portion of the system and the olefinic C−8 proton would represent the X portion.[75] The olefinic proton should appear as a symmetrical quartet with the separation of line 1 and line 4 equal to $J_{AX} + J_{BX}$. Since the olefinic proton does not appear as a quartet but only as a broad singlet, other protons also appeared to be coupled with the olefinic proton. The broadness of the C−9 methyl proton idicated that the C−8 olefinic proton and the C−9 methyl protons were also weakly coupled. Weak coupling of the methyl and olefinic proton in the $-\overset{H_3C}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-$ grouping is also found in compounds such as Δ^3-carene and piperitone,[76] which have a structure similar to ring A of (66).



Carene



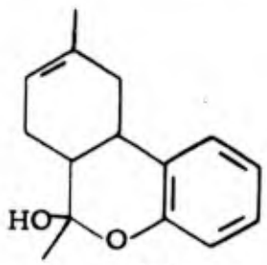
Piperitone

The broad singlet for the olefinic proton in (66) may be explained by the fact that the proton is coupled with the C−7 methylene protons and the C−9 methyl group resulting in a complex multiplet not resolved by the spectrometer.

The assignment of the alicyclic double bond to the 8(9)-position in (66) was based on the spin-spin splitting pattern of the C−10a proton. A signal for the 10a proton appeared as a quartet centered at 3.45 ppm. The splitting pattern was characteristic of the C proton of an ABC system of interacting nuclei.[70, 75] This showed that the 10a proton was adjacent to two

nonequivalent protons. Since no proton was present on 6a, the two protons had to be the pseudoaxial and pseudoequatorial protons of the C−10 methylene group. The presence of a $-\underset{H}{C}=\underset{CH_3}{C}-$ grouping in (66) had shown that one terminus of the alicyclic double bond had to be on C−9. Since the 10a proton was adjacent to a methylene group, the only possible position for the alicyclic double bond in (66) was $\Delta^{8,9}$.

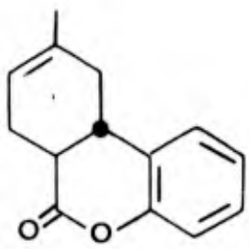
The second step of the proposed synthesis consisted in the hydrolysis and decarboxylation of (66), but this reaction was not successful. Treatment of (66) with aqueous sodium hydroxide under reflux yielded two products. One product, insoluble in the basic hydrolysis medium, was tentatively assigned structure (68) on the basis of the elemental analysis and IR spectrum.



(68)

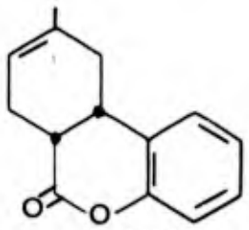
The attempted acid cleavage of the hemiketal (68) to form the ketone (67) was unsuccessful.

The second product formed in the basic hydrolysis of (66) was obtained on acidification of the reaction medium. This material was a white solid, mp 143° to 145°C, identical to the trans-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone (56), mp 143° to 145°C, reported by Strojny and Taylor.[65]



(56)

Mild basic hydrolysis of (66) in 2% methanolic potassium hydroxide yielded a white crystalline product, mp 91° to 93°C, that had an IR spectrum identical to the IR spectrum of an authentic sample of cis-9-methyl-6a, 7, 10, -10a-tetrahydro-6-dibenzopyrone (55).



(55)

The isolation of (55) and (56) from the hydrolysis of (66) placed the methyl group of (66) into the 9 position, since Strojny and Taylor[65] had rigorously proved the position of the methyl group in these compounds.

Since the Diels-Alder addition of a diene to a dieneophile is a cis-addition,[77] the assignment of a cis- relationship between the 6a-acetyl and 10a-hydrogen of (66) is based on the assumption that the cis- relationship of the 3-acetyl group and the hydrogen of the adjacent CH group of 3-acetylcoumarin (65) is retained under the conditions of the Diels-Alder addition of isoprene to (65).

As mentioned above, the hydrolysis products obtained from (66) were identified by comparison with authentic samples of cis- and trans-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone (55) and (56) obtained in a single step by the condensation of 3-carboxycoumarin with isoprene under simultaneous decarboxylation. Strojny and Taylor[65] had obtained (55) and (56) by the hydrolysis and decarboxylation of 6a-carbethoxy-9-methyl-6a, 7, -10, 10a-tetrahydro-6-dibenzopyrone.

The Diels-Alder addition of isoprene to 3-carboxycoumarin took place readily at 180°C to give a crystalline adduct mixture, mp 70° to 130°C. Fractional crystallization of this adduct from ethanol yielded two isomeric compounds. One isomer, mp 94° to 96°C, had an IR spectrum that was identical to the IR spectrum published by Strojny and Taylor[65] for cis-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone. The second isomer, mp 144° to 145°C, showed the IR spectrum of trans-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone recorded by Strojny and Taylor.[65]

The presence of methyl groups attached to unsaturated carbon atoms in the NMR spectra of (55) and (56) was indicated by signals at 1.78 ppm (singlet) in (55) and 1.65 ppm (singlet) in (56) (table III). The presence of only one olefinic proton at 5.57 ppm (broad singlet) in (55) and 5.47 ppm (broad singlet) in (56) places the alicyclic double bond of both compounds in either the $\Delta^{8,9}$ or $\Delta^{9,10}$ positions.

Table III. NMR Spectra of (55) and (56) in $CDCl_3$

Group	Chemical shift from tetramethylsilane	
	(55)	(56)
	ppm	
C−9 methyl	1.78 (singlet)	1.65 (singlet)
Olefinic proton	5.57 (broad singlet)	5.47 (broad singlet)
Aromatic protons	7.21 (multiplet)	7.20 (multiplet)

Since both (55) and (56) were obtained by the mild hydrolysis of (66), which had an alicyclic $\Delta^{8,9}$ bond, the alicyclic double bonds of (55) and (56) were assigned to the same position.

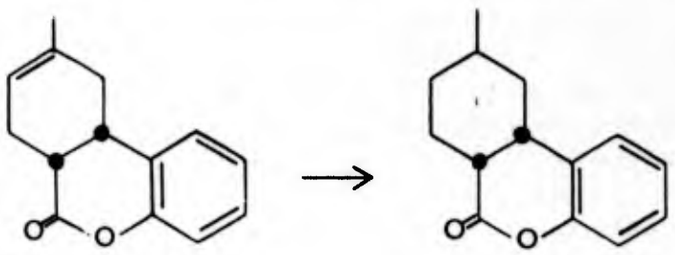
Major differences between (55) and (56) were observed for the signals for the methylene protons at C−7 and C−10 and the methine protons at C−6a and C−10a.

In the instance of the cis-pyrone (55), the C−7 and C−10 methylenes were not equivalent. One of the methylene groups appeared at 2.05 ppm and the other as part of the series of bands between 2.3 and 3.2 ppm where it overlaps with the C−6a and C−10a protons.

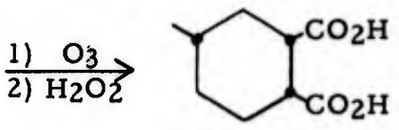
The assignment of cis- and trans- structures to the pyrones (55) and (56) was made by Strojny and Taylor[65] on the basis of the stability of the two isomers. They assumed that the trans-isomer is the most stable form for this type of molecule. They found that the high-melting isomer (56) was the stable form and assigned a trans- configuration to this isomer. The low-melting isomer (55) could be converted to the high-melting isomer (56) by treatment with ammonium acetate at 250°C. On the basis of these data, Strojny and Taylor assigned a cis- configuration to the low-melting isomer (55).

Since the assignment of the configuration of the pyrones (55) and (56) is based on an assumption, it would be of interest to have more definite evidence for the configuration of these compounds. This problem was attacked in a manner suggested by the work of Linstead and coworkers[68] on the stereochemistry of the perhydrophenanthrenes.

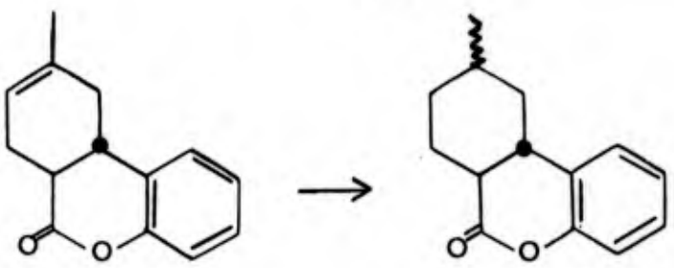
The following outline illustrates the approacn for the elucidation of the stereochemistry of the ring fusion of rings A and B in (55) and (56).



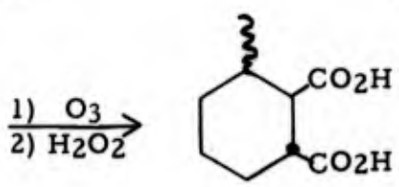
(55) (69)



(70)



(56) (71)

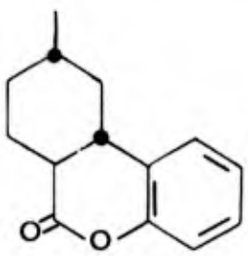


(72)

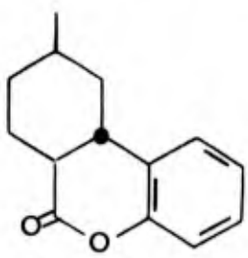
The pyrone (55) was catalytically hydrogenated[26] at room temperature in the presence of platinum oxide catalyst to form the hexahydropyrone (69) in good yield. That reduction had occurred was indicated by a change in the melting point from 94° to 96°C of the starting material (55) to 74° to 76°C for the reaction product. The IR spectrum of the reduced pyrone (69) showed by the presence of aromatic bands at 3085, 3060, 3045, 1610, and 1590 cm^{-1} that the phenyl ring was still intact.

The hexahydropyrone (69) was dissolved in glacial acetic acid and treated with ozone for 4 hr at room temperature. The reaction solution slowly changed from colorless to yellow during this period. The ozonide was then decomposed with aqueous hydrogen peroxide at room temperature for 12 hr. About one-half of the starting material (69) was recovered unchanged. A small amount of a crystalline acid was isolated and identified as cis-4-methyl-cis, cis-hexahydrophthalic acid (70) by its IR spectrum, which was identical to the IR spectrum of an authentic sample.[69] Assuming that no isomerization occurred under the above reaction conditions, the data showed that in order to obtain (70) from (55) by ozonolysis the ring fusion of rings A and B in (55) must be cis-.

The catalytic hydrogenation of the pyrone (56) yielded two products, mp 135° to 137°C (35% of total product isolated) and mp 65° to 67°C (65% of total product isolated). The IR spectra of these two materials were different from those of the starting material (56) and also differed from each other. They showed that the aromatic ring was still intact in both compounds. The two products from the reduction of (56) were assigned structures (73a) and (73b).



(73a)



(73b)

Strojny and Taylor[65] had shown that the addition of hydrogen bromide to (56) yielded a mixture of diastereoisomers, while (55) gave only one isomer under the same conditions. They interpreted their results on the basis of an examination of models of the cis- and trans-pyrones (55) and (56). The models of the cis-pyrone (55) showed that the most stable configuration

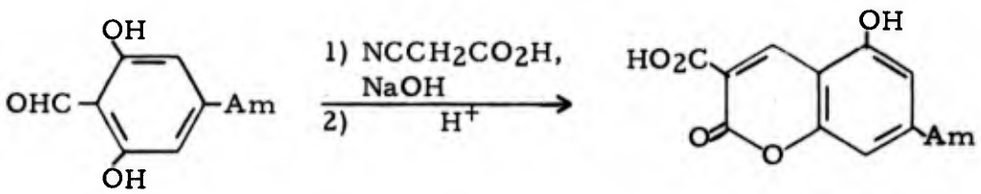
of this compound is one in which the cyclohexene ring is doubled over the phenyl ring to form a V-shaped molecule. The hydrogen bromide can only add to one side of the alicyclic double bond in (55), because the other side was hindered by the phenyl ring.

The Dreiding model of (56) showed that this molecule has an overall planar structure. Attack of the hydrogen bromide can occur theoretically with equal ease from either side of the alicyclic double bond. Although Strojny and Taylor did not attempt to determine the amounts of each of the two diastereomeric bromides formed by the addition of hydrogen bromide to (56), they did show that two diastereomeric bromides are formed.

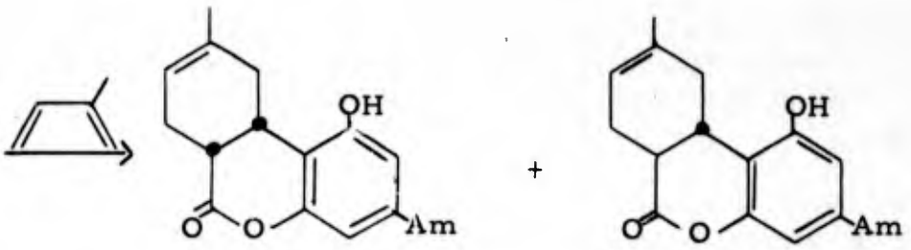
The same arguments can be applied to the catalytic hydrogenation of (55) and (56). The reduction of the cis-pyrone (55) will, therefore, yield only one hexahydro derivative (69). The alicyclic double bond of (55) can only interact with the catalyst from one side, because of the hindrance of the phenyl ring. The planar trans-pyrone (56) can interact with the catalyst from either side of the double bond and form both of the two possible hexahydropyrones (73a) and (73b).

Treatment of a mixture of (73a) and (73b) with ozone did not proceed as smoothly as did compound (69). These two compounds were much more sensitive to the attack of ozone and yielded mostly tars that could not be purified. The ozonolysis of the mixture of (72) and (73), however, yielded a very small amount of an acid that was identified as trans-4-methyl-cis, trans-hexahydrophthalic acid from its IR spectrum. These data showed that the pyrone (56) must have a trans- configuration of the fusion of rings A and B.

The results obtained in the synthesis of the model compounds (66), (56), and (55) suggested two possible routes (schemes II and III) for the synthesis of the two diastereomeric cis- and trans-tetrahydrocannabinols with a $\Delta^{8,9}$-alicyclic double bond in the same position as the alicyclic double bond of one of the naturally occurring tetrahydrocannobinols.

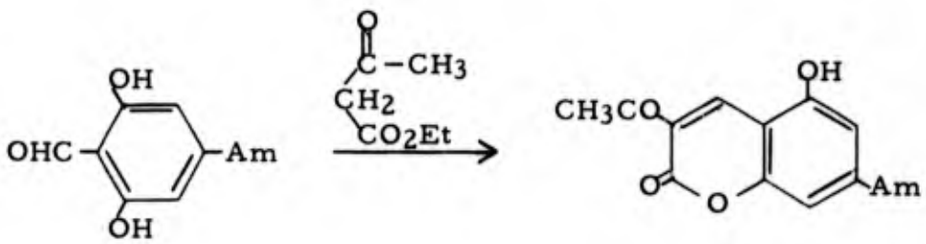


(74) (75)

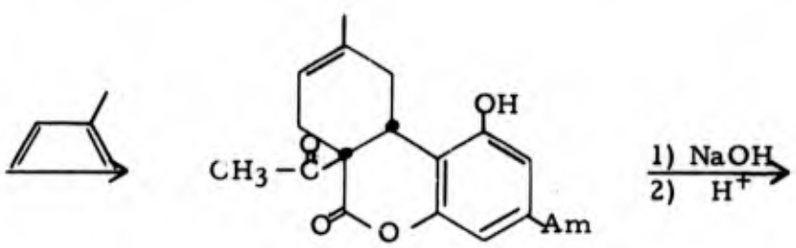


(76) (77)

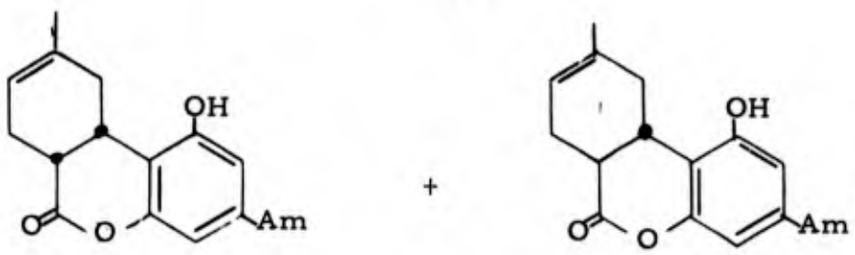
Scheme II



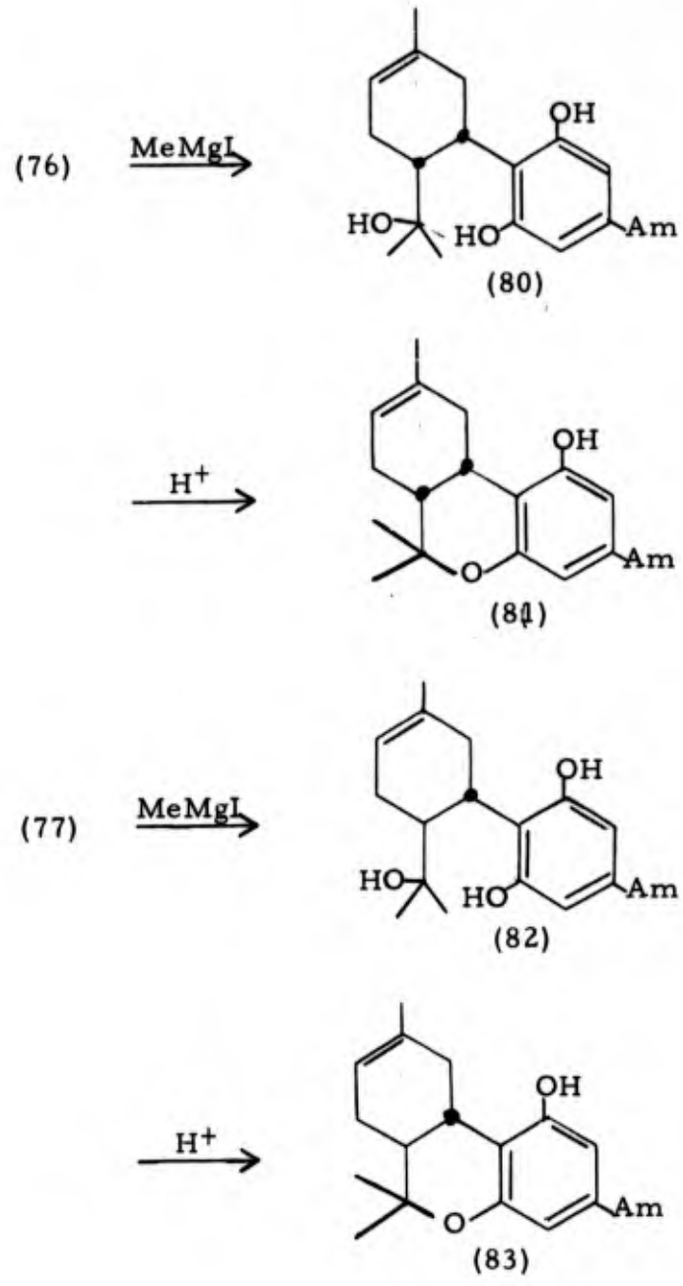
(74) (78)



(79)



(76) (77)



Scheme III

The synthesis of the cis- and trans-pyrones (76) and (77), the precursors for the cis- and trans-tetrahydrocannabinols (81) and (83), was first attempted by the method outlined under scheme II. 2,6-Dihydroxy-4-n-amylbenzaldehyde (74) was condensed with cyanoacetic acid in the presence

of aqueous sodium hydroxide.[63] Acid hydrolysis of the intermediate coumarin-imide[78] yielded the 3-carboxy-5-hydroxy-7-n-amylcoumarin (75), mp 199° to 200°C. The IR spectrum of (75) showed bands at 1725 (carbonyl), 3290 (hydroxyl), and 3300 to 2400 cm^{-1} (hydroxyl of carboxyl group). The elemental analysis and the IR spectrum demonstrated that the desired compound (75) had been formed.

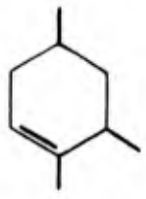
The Diels-Alder addition of isoprene to (75) yielded a single crystalline adduct melting at 123° to 125°C, to which structure (76) was assigned. The IR spectrum of (76) showed the presence of a hydroxyl absorption at 3420 cm^{-1} and a lactone carbonyl at 1745 cm^{-1}. The UV spectrum (76) had maxima at 283 (ε 2520) and 277 mμ (ε 2250), which established that the alicyclic double bond of (76) was not in conjugation with the phenyl nucleus.

The signal for a methyl group attached to a saturated carbon atom at 0.88 ppm (triplet) was assigned to the methyl of the n-amyl group in (76) (table IV). The broad singlet for one proton at 5.45 ppm was assigned to an olefinic proton.

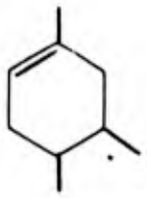
Table IV. NMR Spectrum of (76) in $CDCl_3$

Group	Chemical shift from tetramethylsilane
	ppm
Methyl of n-amyl	0.88 (triplet)
C−9 methyl	1.61 (broad singlet)
C−10a proton	3.45 (five lines)
Aromatic protons	5.47 (two protons)

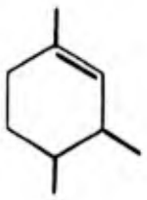
The presence of only one olefinic proton and the fact that the double bond was not conjugated with the phenyl nucleus reduced the number of possible positions for the alicyclic double bond in the adduct to the three represented by the following partial structures.



Δ-6a, 7



Δ-8, 9



Δ-9, 10

The broad singlet at 1.62 ppm for C−9 methyl of (76) eliminated a $\Delta^{6a,7}$ position for the alicyclic double bond, since the methyl of such a compound would be split into a doublet by the methine at C−9. Therefore, the methyl of the cyclohexene ring in (76) must be part of a $-\underset{H}{C}=\underset{CH_3}{C}-$ group. The chemical shift of the methyl signal was also in agreement with this assignment. The above data showed that the alicyclic double bond of (76) had one terminus on C−9. The alicyclic double bond was assigned to the $\Delta^{8,9}$ position by the appearance of the spin-spin splitting pattern exhibited by the 10a proton. The signal for the 10a proton appeared as five lines at 3.50 ppm. This indicates that the 10a proton must be adjacent to a methylene group at C−10 and a methine at C−6a. If the double bond were in the $\Delta^{9,10}$ position, the 10a proton would show a maximum of four lines, assuming the 10a proton were coupled with both the 6a proton and a C−10 olefinic proton to form an AMX system.[75] Since a more complex splitting pattern is observed for the 10a proton, the alicyclic double bond is placed in the $\Delta^{8,9}$ position. More proof for the $\Delta^{8,9}$ position for the alicyclic double bond will be given in the later discussion of the NMR spectrum of (79).

Since the addition of isoprene to (75) yielded only one of the possible pyrones (76) or (77), the next step was to prove the stereochemistry of the fusion of rings A and B in the adduct. The adduct was catalytically hydrogenated at room temperature in the presence of a platinum oxide catalyst to form only one hexahydropyrone, mp 136° to 137°C. The elemental analysis and IR spectrum of this material demonstrated that reduction of the alicyclic double bond had occurred, but that the phenyl ring had not been disturbed. The structure of 1-hydroxy-3-n-amyl-9-methyl-6a,7,8,9,10,10a-hexahydro-6-dibenzopyrone was assigned to the reaction product.

The 1-hydroxy-3-n-amyl-9-methyl-6a,7,8,9,10,10a-hexahydro-6-dibenzopyrone was treated with ozone at room temperature for 4 hr. The ozonide was decomposed with aqueous hydrogen peroxide to yield an acid, mp 172° to 174°C. The IR spectra of this acid and of an authentic sample of cis-4-methyl-cis,cis-hexahydrophthalic acid (70) were identical. Assuming that no isomerization occurred under the above reaction conditions, the adduct obtained from the addition of isoprene to 3-carboxy-5-hydroxy-7-n-amyl-coumarin (75) must be cis-1-hydroxy-3-n-amyl-9-methyl-6a,7,10,10a-tetrahydro-6-dibenzopyrone (76).

The sulfur dehydrogenation of (76) yielded a product identical to an authentic sample of 1-hydroxy-3-n-amyl-9-methyl-6-dibenzopyrone. The acetate of the dehydrogenation product of (76) was also identical to an authentic sample of 1-acetoxy-3-n-amyl-9-methyl-6-dibenzopyrone. Comparison of

the dehydrogenation product of (76) with a sample of 1-hydroxy-3-n-amyl-8-methyl-6-dibenzopyrone showed the nonidentity of these materials. Since isoprene is an unsymmetrical diene, its condensation with (75) could lead to the formation of either 1-hydroxy-3-n-amyl-8-methyl or 9-methyl-6a,-7, 10, 10a-tetra-6-dibenzopyrone or both. The above data, however, clearly indicated the exclusive formation of the 9-methyl isomer (76).

The next step of this investigation was the attempted preparation of trans-1-hydroxy-3-n-amyl-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone (77). Since the addition of isoprene to (75) yielded only the cis-pyrone (76), the procedure outlined under III was tried.

Ethyl acetoacetate was condensed with 2, 6-dihydroxy-4-n-amylbenzaldehyde (74) in the presence of piperidine to yield 3-acetyl-5-hydroxy-7-n-amylcoumarin (78), mp 273° to 275°C. The Diels-Alder addition of isoprene to (78) yielded a crystalline adduct (79), mp 173° to 175°C, in 80% yield. Two different carbonyl bands at 1750 and 1705 cm^{-1} in the IR spectrum of the adduct indicated the presence of a lactone and a keto group in (79). A hydroxyl band was also observed at 3390 cm^{-1}. The UV spectrum of (79) had maxima at 285 (ε 2820) and 280 mμ (ε 2570). This established that the alicyclic double bond of the adduct was not conjugated with the phenyl ring.

The NMR spectrum of (79) obtained by the addition of isoprene to (78) showed the presence of three methyl groups (table V). A signal at 0.87 ppm (triplet) was due to the methyl of the n-amyl group. The signal at 2.10 ppm (sharp singlet) was due to the methyl of the acetyl functional group. The signal of the other methyl group at 1.65 ppm (singlet) indicated that this methyl was part of a $-\underset{H_3C}{C}=\underset{H}{C}-$ group. These data placed the alicyclic double bond of the adduct in either the $\Delta^{8,9}$ or $\Delta^{9,10}$ position. A signal at 5.47 ppm (broad singlet) for one olefinic proton added further support to this assignment. The signal for the 10a proton appears as four lines at 3.87 ppm. Such a splitting pattern is characteristic of the ABC-type system.[70, 75] These data placed the alicyclic double bond in the $\Delta^{8,9}$ position. If the double bond were in the $\Delta^{9,10}$ position, the signal for the 10a proton would be a doublet caused by the coupling of the singlet olefinic proton at C−10 with the 10a proton. Since the 10a proton must be adjacent to a methylene group and one terminus of the alicyclic double bond is on C−9, the only possible position for the alicyclic double bond is $\Delta^{8,9}$.

Basic hydrolysis of (79) in refluxing aqueous sodium hydroxide yielded only the cis-pyrone (76). Hydrolysis of (79) at room temperature in ethanolic potassium hydroxide also yielded exclusively (76).

Table V. NMR Spectrum of (79) in $CDCl_3$

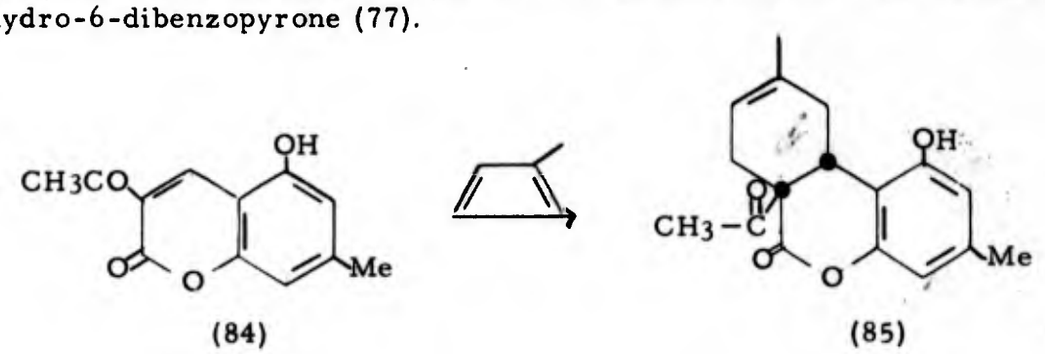
Group	Chemical shift from tetramethylsilane
	ppm
CH_3 of n-amyl	0.87 (triplet)
CH_3 of acetyl	2.10 (sharp singlet)
CH_3 at C−9	1.65 (singlet)
C−10a proton	3.87 (quartet)
Olefinic proton	5.47 (broad singlet)
Aromatic protons	6.45 (two protons)

Since the mild basic hydrolysis of (79) under conditions that should not cause the isomerization of the alicyclic double bond yielded the same pyrone as obtained from the addition of isoprene to (75), this result added support to the assignment of the alicyclic double bond to the $\Delta^{8,9}$ position in (76).

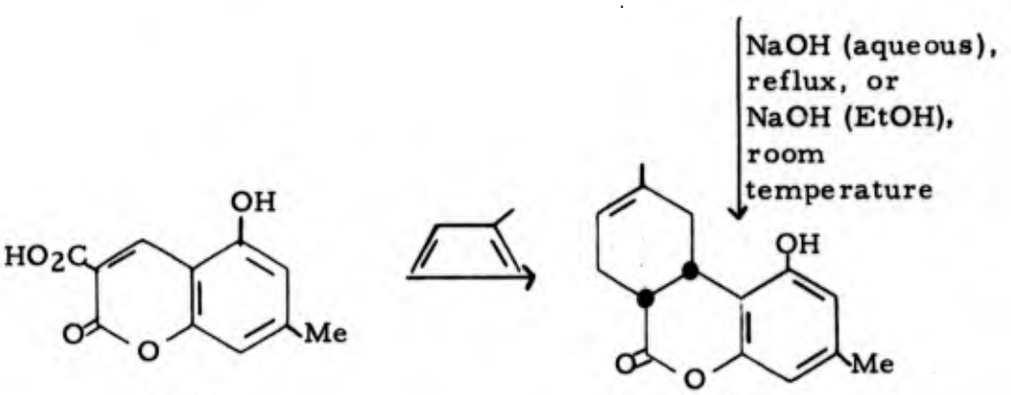
The addition of isoprene to 3-carboxycoumarin yielded the two possible diastereomeric cis- and trans-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrones (55) and (56). The addition of isoprene, however, to a 3-carboxycoumarin that has a hydroxyl group in the 5 position and an n-amyl group in the 7 position yielded only one of the two possible diastereomeric isomers, cis-1-hydroxy-3-n-amyl-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone (76). Also, the hydrolysis of cis-9-methyl-6a-acetyl-6a, 7, 10, -10a-tetrahydro-6-dibenzopyrone (66) yielded the two diastereomeric cis- and trans-pyrones (55) and (56), but the hydrolysis of a cis-9-methyl-6a-acetyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone that has a hydroxyl group in the 1 position and an n-amyl group in the 3 position yielded only one of the two possible diastereomeric isomers, cis-1-hydroxy-3-n-amyl-9-methyl-6a, 7, -10, 10a-tetrahydro-6-dibenzopyrone (76). Very mild hydrolysis of (66) yielded the cis-pyrone (55), but under more vigorous conditions the trans-pyrone (56) was obtained. In the instance of (79), however, both mild or vigorous hydrolysis yielded only the cis-pyrone (76). The evidence obtained by Strojny and Taylor[65] showed that the cis-pyrone (55) could be isomerized to the more stable trans-pyrone (56) but not vice versa. The hydrolysis data for (66) are in line with the results reported by Strojny and Taylor. From the data obtained, it appeared

that, concerning the pyrones with a 1-hydroxy and a 3-n-amyl group, the cis- isomer rather than the trans- isomer was the more stable isomer.

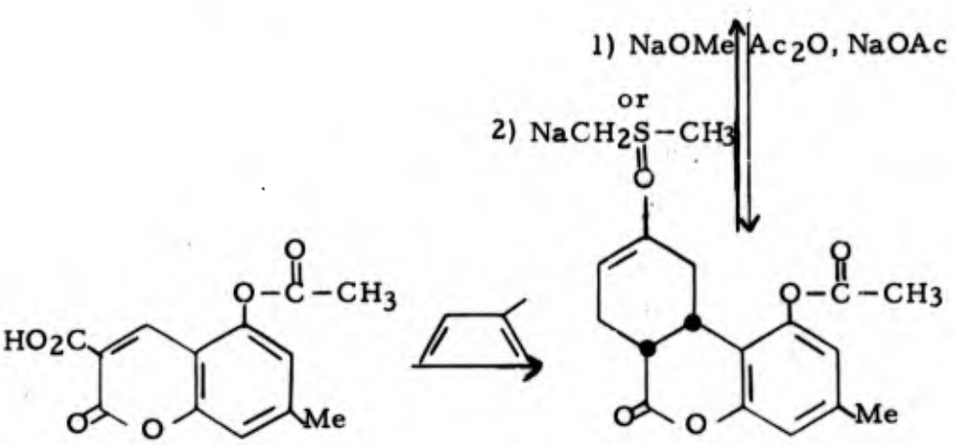
An attempt was made to find a synthetic method that could be used for the synthesis of the trans-1-hydroxy-3-n-amyl-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone (77).



(84) (85)



(86) (87)



(88) (89)

The methyl isomer in place of the n-amyl isomer was used as a model system because of the availability of the starting material, 2,6-dihydroxy-4-methylbenzaldehyde. The 3-acetyl-5-hydroxy-7-methylcoumarin (84) was prepared by the piperidine-catalyzed condensation of ethyl acetoacetate with 2,6-dihydroxy-4-methylbenzaldehyde. Isoprene was condensed with (84) to yield the adduct (85). Two carbonyl bands at 1755 and 1700 cm^{-1} and a hydroxyl band at 3400 cm^{-1} were observed in IR spectrum of (85). Maxima at 284 (ε 2390) and 278 mμ (ε 2350) in the UV spectrum established that the alicyclic double bond of (85) was not conjugated with the phenyl nucleus. The NMR spectrum showed the presence of a methyl group attached to an unsaturated carbon at 1.69 ppm and two methyl groups at 2.12 and 2.25 ppm for the methyl group attached to the phenyl ring and the acetyl methyl group. A signal for an olefinic proton was present at 5.45 ppm (broad singlet). The 10a proton appeared as four lines at 3.80 ppm. This was in agreement with the NMR data obtained for the alicyclic double bond in the $\Delta^{8,9}$-position for reasons described previously for (66) and (79).

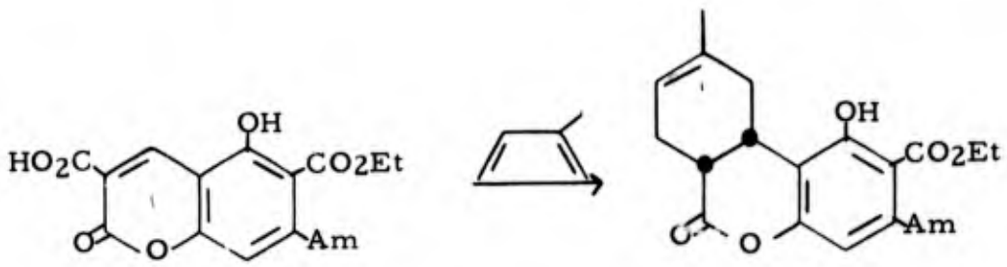
The basic hydrolysis of (85) in either refluxing aqueous sodium hydroxide or in methanolic potassium hydroxide at room temperature yielded the same compound, mp 232° to 234°C, in both instances. This compound was identified as cis-1-hydroxy-3,9-dimethyl-6a,7,10,10a-tetrahydro-6-dibenzopyrone (87) on the basis of the NMR spectrum. The positions and splitting patterns were the same for the 9-methyl group, 8-olefinic proton, the 10a proton, and the methylene groups of the cyclohexene ring as for the cis-1-hydroxy-3-n-amyl-9-methyl-6a,7,10,10a-tetrahydro-6-dibenzopyrone (76). Since the NMR spectrum of the cis- and trans-pyrones (55) and (56) were shown to differ greatly from each other, it seems permissible to assign a cis- ring fusion to rings A and B of (87) on the basis of the similarity between the spectra of (87) and (76).

The Diels-Alder addition of isoprene to 3-carboxy-5-hydroxy-7-methylcoumarin (86) yielded (87), which was identified by its IR spectrum and melting point.

In order to determine if a protective group on the hydroxyl group of the coumarin (86) would affect the stereochemistry of the ring fusion of rings A and B in (87), the coumarin (86) was acetylated with isopropenyl acetate using p-toluenesulfonic acid as the catalyst to yield 3-carboxy-5-acetoxy-7-methyl coumarin (88). The addition of isoprene to (88) yielded 1-acetoxy-3,9-dimethyl-6a,7,10,10a-tetrahydro-6-dibenzopyrone (89). The IR and NMR spectra of (89) were identical to the spectra of the compound obtained by the acetylation of (87). This showed that the ring fusion of the adduct of isoprene and (88) also is cis.

Attempts to isomerize (87) or (89) to the trans- isomers were not successful. The pyrone (89) was treated with sodium methylsulfinyl carbanion in dry dimethylsulfoxide at 50°C for 4 hr to yield (87). Similarly, treatment of (89) with sodium methoxide in refluxing xylene yielded only (87). The pyrone (87) was heated with ammonium acetate at 215°C for 1 hr to yield only starting material. cis-1-Hydroxy-3-n-amyl-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone (76) remained similarly unaffected by sodium methoxide or ammonium acetate.

In another attempt to obtain trans-1-hydroxy-3-n-amyl-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone (77) from a Diels-Alder addition of isoprene to a carboxycoumarin, isoprene was condensed with 3-carboxy-5-hydroxy-6-carbethoxy-7-n-amyl-coumarin (90) to yield only one crystalline adduct (91), mp 70° to 72°C.



(90) (91)

The NMR data showed that the alicyclic double bond of (91) was in the $\Delta^{8,9}$ position for reasons previously described (table VI).

The basic hydrolysis of (91) yielded only highly colored tars that could not be purified. The treatment of (91) with hydrochloric acid in aqueous dioxane gave a crystalline solid, mp 146° to 147°C, the NMR spectrum of which showed no signal for an olefinic proton, indicating that the alicyclic double bond was no longer present in the hydrolysis product. The NMR spectrum also showed that the carbethoxy group was still intact. The mass spectrum and elemental analysis of the hydrolysis product proved that the elements of water had been added to (91). The desired trans-1-hydroxy-3-n-amyl-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone, therefore, cannot be obtained by the acid hydrolysis of (91).

Table VI. NMR Spectrum of (91) in $CDCl_3$

Group	Chemical shift from tetramethylsilane
	ppm
CH_3 of n-amyl	0.89 (triplet)
CH_3 at C−9	1.65 (singlet)
CH_3 of carboethoxy	1.41 (triplet, J = 7 cps)
CH_2 of carboethoxy	4.47 (quartet, J = 7 cps)
C−10a proton	3.55 (quintet)
Olefinic proton	5.47
Aromatic protons	6.48

Since the Diels-Alder addition of a diene to a dieneophile is a cis-addition, the pyrones obtained from the addition of isoprene to a 3-substituted coumarin must have an initial cis- configuration. The fact that the addition of isoprene to a 3-carboxycoumarin without substituents on the phenyl ring yielded both the cis- and trans-pyrones (55) and (56) showed that the initially formed cis-pyrone must undergo epimerization under the conditions of the Diels-Alder reaction. Also, the vigorous hydrolysis of cis-9-methyl-6a-acetyl-6a, 7, 10, -10a-tetrahydro-6-dibenzopyrones proceeds with isomerization to (56).

The failure to obtain a pyrone with trans- fusion of the cyclohexene ring A and the lactone ring B by the Diels-Alder addition of isoprene to a 3-carboxy-5-hydroxy-7-alkylcoumarin or by the hydrolysis of a 1-hydroxy-3-alkyl-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone cannot be explained satisfactorily at the present time. This failure might be caused by both the alkyl group and hydroxyl group. The data did show, however, that the type of alkyl group is not of primary importance, since both the methyl- and n-amyl-substituted compounds gave the same results. Because the position of the alkyl group excludes any steric interactions, the effect that influences the stereochemistry of the ring fusion in the pyrone can only be inductive.

To clarify the mechanism of the formation of a trans-pyrone from the initial cis- adduct, a more careful study would be required.

The cis-1-hydroxy-3-n-amyl-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone (76) was converted to a tetrahydrocannabinol by treatment with an 8-M excess of methylmagnesium iodide in refluxing benzene. The yellow resin obtained was purified by column chromatography on silica gel to yield a crystalline compound, mp 118° to 119°C. A very strong hydroxyl absorption band in the IR spectrum indicated the presence of more than one hydroxyl group. Treatment of the reaction product with ethanolic potassium hydroxide produced an intense blue color characteristic of a resorcinol derivative. The UV spectrum had the same absorption maxima at 275 (ε 1506) and 283 mμ (ε 1520) as (76). Therefore, migration of the double bond had not occurred under the conditions of the Grignard reaction. Based on the above data, the product appeared to be 1-methyl-5-(2, 6-dihydroxy-4-n-amylphenyl)-4-(α-hydroxyl-α-methylethyl)-1-cyclohexene (80). Strojny and Taylor[65] had shown that the treatment of the cis- and trans-pyrones (55) and (66) with methylmagnesium iodide yielded the cis- and trans-1-methyl-5-(o-hydroxyphenyl)-4-(α-hydroxy-α-methylethyl)-1-cyclohexenes. The structure assigned to the methylation product (80) is in agreement with the results of Strojny and Taylor.[65]

Treatment of (80) with sulfuric acid in methanol for 24 hr at room temperature yielded a brown resin. Analysis of this resin by TLC on silica gel using benzene as the developer indicated that the resin was composed of two major components. The resin was chromatographed on a column packed with silica gel using benzene as the eluent. One of the fractions eluted from the column was a white solid, mp 68° to 72°C. On the basis of its IR spectrum, NMR spectrum, and elemental analysis, which proved that this compound was not the tetrahydrocannabinol (81), it was tentatively identified as a methoxy derivative of (80).

The other fraction eluted from the silica gel column was a yellow resin that accounted for about 65% of the total material recovered. The IR, UV, and NMR spectra of this resin were consistent with a tetrahydrocannabinol structure. TLC on silica gel-silver nitrate showed the presence of at least three components that were separated preparatively on a column packed with silica gel-silver nitrate. The fastest moving zone yielded a small amount of a white solid, mp 108° to 109°C, upon crystallization from hexane. This material was not identified. The second compound eluted from the column accounted for 88% of the total amount of material recovered from the column. This compound crystallized from a hexane solution after standing in the cold for several weeks to yield white rosettes, mp 64° to 65°C. The third component eluted from the column was crystallized from hexane to yield 3 mg of white needles, mp 50° to 53°C. The structure of this compound is not

certain, but the IR and UV spectra did indicate that that this material may be a double-bond isomer of the material melting at 64° to 65°C.

The major component, mp 64° to 65°C, obtained from the silica gel-silver nitrate column was assigned structure (81) on the basis of the IR, UV, and NMR spectra (table VII). The UV spectrum of (81) had adsorption maxima at 283 (ε 1210), 275 (ε 1170), and 212 mμ (ε 39,300), which showed that the alicyclic double bond was not conjugated with the phenyl nucleus. The IR spectrum of (81) showed the presence of a hydroxyl group, a gem-dimethyl group, and an aromatic ring.

Table VII. NMR Spectrum of (81) in $CDCl_3$

Group	Chemical shift from tetramethylsilane
	ppm
CH_3 of n-amyl	0.88 (triplet, J = 6 cps)
CH_3 at C−9 and $2CH_3$'s at C−6	1.35, 1.67, 1.95 (singlets)
C−10a proton	≈2.50
Olefinic proton	4.20
Aromatic protons	6.18 (doublet, J = 2 cps) 6.37 (doublet, J = 2 cps)

The NMR spectrum of (81) showed the presence of two nonequivalent aromatic protons coupled to each other with a coupling constant and an AB splitting pattern characteristic of nonequivalent meta protons. This indicated that a pyran ring was present in (82). The presence of three deshielded methyl groups, all of which appeared as singlets in the NMR spectrum, showed that the alicyclic double bond of (81) must have one terminus of the double bond at C−9. Two of these methyl groups are at C−6, α to the pyran oxygen, and the other methyl group is at C−9. If the alicyclic double bond in (81) did not have one terminus at C−9, methyl would have appeared as a doublet (table VII) upfield from the position of any of the above methyl groups.

A signal at 4.20 ppm as a distorted triplet (J = 3 cps) was assigned to the olefinic proton. This is a high field position for an olefinic proton, but the Dreiding models of (81) showed that the cyclohexene ring is folded over the phenyl ring, which placed the olefinic proton above the plane of the phenyl ring where it could be shielded by the ring currents of the phenyl ring.[73,79] A doublet (J = 3 cps) with an area for two protons was observed at 1.80 ppm. These data indicated that the olefinic proton was coupled to an adjacent methylene group. The dihedral angle between the methylene protons and the olefinic protons was about 55°. The observed coupling constant is of the right magnitude for such angles.[73] Since one terminus of the double bond is on C−9, the alicyclic double bond of (81) must be in the 8(9)-position, because this is the only position in which the olefinic proton is adjacent to a methylene group.

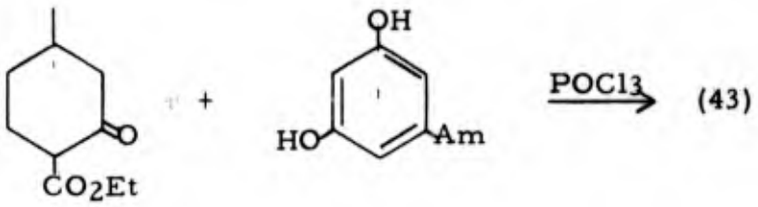
The assignment of a cis- ring fusion of the cyclohexene and pyran rings of (81) is substantiated by the work of Strojny and Taylor[65] on the methylation of cis- and trans-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrones to yield the cis- and trans-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrans, respectively. Since the pyrone (76) was shown to have a cis- configuration, the pyran (81) prepared by the methylation of (76) must also have by analogy a cis- configuration.

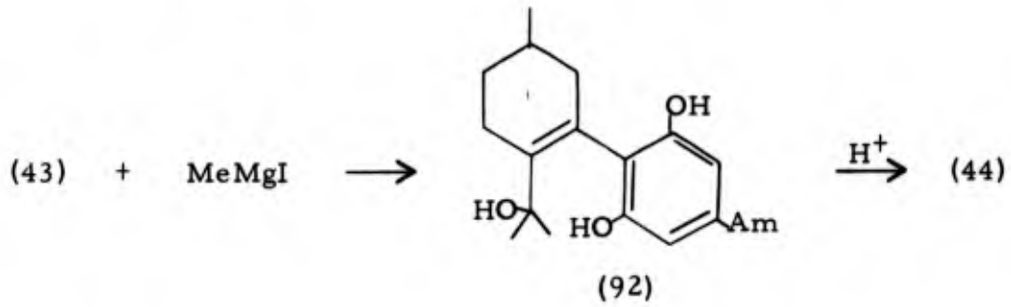
The catalytic hydrogenation of (81) offered additional proof that this tetrahydrocannabinol has a cis- configuration. Hydrogenation of (81) at room temperature using a platinum oxide catalyst yielded only one dihydro derivative, a crystalline solid, mp 75° to 76°C. As discussed previously, the catalytic reduction of the cis-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone and cis-1-hydroxy-3-n-amyl-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone yielded only one dihydro derivative. On the other hand, the reduction of trans-9-methyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyrone yielded two dihydro derivatives. This was explained by the fact that, in the instance of the cis-pyrones, the cyclohexane ring is folded over the phenyl ring and one side of the alicyclic double bond is hindered. Approach of the reagent adding to the double bond can only occur from one side. Therefore, only one of the two possible diastereomeric isomers is formed. Since the trans-pyrone has an overall planar configuration and the alicyclic double is not hindered, approach of the reagent adding to the double bond can occur from either side. The catalytic reduction of (81) yielded only one dihydro derivative, since the double bond appears to be hindered on one side. Therefore, (81) must have a cis- configuration of the ring fusion of cyclohexene ring and pyran ring.

Sulfur dehydrogenation of (81) yielded a compound that TLC on silica gel-silver nitrate showed to be identical to an authentic sample of cannabinol. The dehydrogenation product was converted to an acetate derivative

with acetic anhydride to yield a crystalline compound identical to an authentic sample of cannabinol acetate. These results defined the carbon skeleton of (81).

A second synthetic tetrahydrocannabinol was prepared by the method of Adams[48] as illustrated in the following scheme.





Olivetol was condensed with ethyl 5-methylcyclohexanone-2-carboxylate to yield the pyrone (43), mp 177° to 179°C. Treatment of (43) with methylmagnesium iodide yielded the intermediate triol (92). This compound was not isolated, but was detected by TLC. Treatment of (92) with 10% aqueous sulfuric acid led to the tetrahydrocannabinol (44). Comparative thin-layer and paper chromatographic analysis of the crude tetrahydrocannabinol obtained from the acid treatment of (92) with the two crystalline tetrahydrocannabinols, mp 62° to 63°C and mp 128°C, isolated by Korte and Sieper[44] from a similar preparation, showed that the crude (44) contained only the low-melting isomer. The crude product was purified by column chromatography on silica gel to yield a pale-yellow resin. A solution of this resin in petroleum ether was cooled to -5°C for 7 days. Crystallization began after 4 days and appeared to be complete after 7 days. The white crystalline solid that was isolated had a melting point of 67° to 68°C. The UV spectrum of (44) had adsorption maxima at 277 (ε 11,700) and 229 mμ (ε 27,200), which is indicative of a double bond in conjugation with the phenyl nucleus.

The NMR spectrum of (44) demonstrated the presence of four methyl groups (table VIII). A signal at 0.91 ppm (triplet) was assigned to the methyl group of the n-amyl group and the signal at 1.05 ppm (doublet, J = 6 cps) to the methyl group attached to C−9. Two signals for the gem-dimethyl group appeared as singlets at 1.27 and 1.45 ppm. , No signal for an olefinic proton was observed in the NMR spectrum of (92). This observation places the alicyclic double bond in the $\Delta^{6a, 10a}$ position. If the double bond were in the alternative $\Delta^{10, 10a}$ position conjugated with the phenyl ring, a signal would be observed for an olefinic proton. The existence of the pyran ring in (44) is indicated by the nonequivalence of the two phenyl protons. The signals for the phenyl protons occur at 6.37 ppm (doublet, J = 2 cps) and 6.18 ppm (doublet, J = 2 cps). The NMR spectrum definitely establishes that (44) is 1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-7, 8, 9, 10-tetrahydro-6-dibenzopyran.

Table VIII. NMR Spectrum of (44) in $CDCl_3$

Group	Chemical shift from tetramethylsilane
	ppm
CH_3 of n-amyl	0.91 (triplet)
CH_3 at C−9	1.05 (doublet, J = 2 cps)
CH_3's at C−6	1.27, 1.45 (singlets)
Aromatic protons	6.18 (doublet, J = 2 cps) 6.37 (doublet, J = 2 cps)

VI. STUDIES OF THE PHENOLIC COMPONENTS PRESENT IN PETROLEUM ETHER EXTRACTS OF MARIJUANA.

Work on the marijuana extracts was performed to isolate pure samples of tetrahydrocannabinol and cannabidiolic acid. The five samples of marijuana investigated were grown in eastern Maryland, West Virginia, Mexico, and Spain.

The sample of marijuana obtained from eastern Maryland was several months old when the investigations were conducted. The petroleum ether extract of a 108-gm sample of this marijuana was washed with base to yield 0.08 gm of a base-soluble fraction. TLC of the fraction showed the

presence of several components. The IR spectrum of the base-soluble fraction was similar to that of cannabidiolic acid published by Schultz and Haffner.[33] The UV spectrum showed maxima at 304 (ε 4090), 263 (ε 10,200), and 220 mμ (ε 23,700), which indicated the presence of an α-resorcylic acid. The cannabidiolic acid isolated by Schultz and Haffner[33] had absorption maxima at 315 (ε 2500), 273 (ε 14,000), and 217 mμ (ε 19,500). Since the spectral data of the base-soluble substance were in fair agreement with the spectral data of Schultz and Haffner[33] for cannabidiolic acid, this substance was assumed to be an impure sample of cannabidiolic acid.

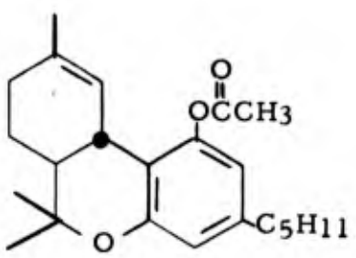
The petroleum ether extract of the eastern Maryland marijuana that had been washed with base was chromatographed on 500 gm of Woelm neutral alumina to yield a solid hydrocarbon and 2.7 gm of a red resin. The resin was analyzed by TLC and was shown to contain a tetrahydrocannabinol and small amounts of cannabidiol and cannabinol. This resin was chromatographed twice on silica gel columns to yield 0.9 gm of a pale-yellow resin. A petroleum ether solution of this resin yielded 10 mg of a white solid, mp 86° to 88°C, after standing at -5°C for 3 wk. Repeated recrystallization of this solid did not result in any change of the melting point. TLC of this solid on silica gel-silver nitrate showed that it was composed of mostly a crystalline tetrahydrocannabinol, mp 143° to 144°C, contaminated with a small amount of two other tetrahydrocannabinols present in the resin from which it was derived. The IR spectrum of the solid, mp 86° to 88°C, was similar to the IR spectrum of the resin from which it was isolated. The UV spectrum had maxima at 284 (ε 1240), 276 (ε 1210), 230 (ε 9900), and 212 mμ (ε 41,000). The UV spectrum of the resin had absorption maxima at 258 (ε 16,000), 277 (ε 1840), and 284 mμ (ε 1920). The UV spectra of the crystalline and noncrystalline tetrahydrocannabinols showed that the two materials were not identical, but both appeared to be tetrahydrocannabinols in which the alicyclic double bond was not conjugated with the phenyl nucleus.

A larger sample of marijuana from eastern Maryland was extracted and treated in the manner described above. In this instance, none of the crystalline compound could be isolated. The resins from the two extractions were combined and chromatographed twice on silica gel. The bulk of the material recovered contained two components by TLC. In addition, a small amount of a resin was recovered that appeared to be homogeneous by thin-layer and paper chromatography. The UV spectrum of this material showed absorption maxima at 209 (ε 37,600), 277 (ε 1330), and 284 mμ (ε 1370). The rotation of this material, $[\alpha]_D^{27}$ -167°, compares favorably with the rotation of $[\alpha]_D$ -161° for a tetrahydrocannabinol isolated by DeRopp.[46]

The material that was still a mixture of tetrahydrocannabinols was separated into three components by chromatography on silica gel-silver nitrate. The major component, which accounted for 87% of the total material recovered, was identical to the tetrahydrocannabinol with a rotation of $[\alpha]_D^{27}$ -167°. A second component, which accounted for 12% of the total amount of material recovered, had a rotation of $[\alpha]_D^{27}$ -260°. The UV spectrum of this material had absorption maxima at 283 (ε 1390), 276 (ε 1330), and 209 mμ (ε 41,000). A third material, which accounted for 1% of the total material recovered, was crystallized from petroleum ether (30° to 60°C) to yield a solid, mp 143° to 145°C. The UV spectrum of this material showed absorption maxima at 212 (ε 49,600), 230 (ε 17,300), 276 (ε 1680), 284 (ε 1750), and 299 mμ (ε 480). The melting point, UV spectrum, and TLC data for this solid showed that it is identical to a crystalline compound isolated by Korte and Sieper[44] from Cannabis sativa. These investigators indicated that this material is a tetrahydrocannabinol but did not elucidate its structure because of the small amount of material obtained. Since only a very small amount of this material was isolated from marijuana, no work on the structure of this material was conducted.

The noncrystalline tetrahydrocannabinol, $[\alpha]_D$ -167°, was shown to be identical to one of the tetrahydrocannabinols present in the distilled "red oil" from Egyptian hashish by IR, UV, and NMR spectra and by TLC. The NMR spectrum (table IX) showed one methyl group attached to an aliphatic carbon at 0.88 ppm (triplet). Three other methyl peaks appear at 1.07, 1.40, and 1.67 ppm. Two of these methyl signals result from the gem-dimethyl group of the pyran ring. The other methyl signal is due to the methyl group at C−9. Signals at 6.37 ppm (singlet) for one olefinic proton and at 3.20 ppm (doublet, J = 10 cps) for the 10a proton were also observed in the NMR spectrum. These data were in agreement with the NMR data reported by Gaoni and Mechoulan[47] for a tetrahydrocannabinol to which they assigned structure (42).

Additional support for $\Delta^{9,10}$ position for the alicyclic double bond in (42) was obtained from the NMR spectrum of the acetate ester (42a) of (42), obtained by treatment of (42) with acetic anhydride in pyridine.



(42a)

Table IX. NMR Spectrum of (42) in $CDCl_3$

Group	Chemical shift from tetramethylsilane
	ppm
CH_3 of n-amyl	0.88 (triplet)
CH_3 at C−6	1.67 (slightly broader singlet)
CH_3's at C−6	1.07, 1.40 (singlets)
C−10a proton	3.20 (doublet, J = 8 cps)
Olefinic proton	6.37 (broad singlet)
Aromatic protons	6.13 (doublet, J = 2 cps) 6.30 (doublet, J = 2 cps)

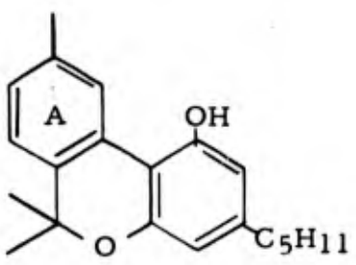
The most important difference between the NMR spectra of (42) and (42a) was the position of the signal for the olefinic proton (table X). In (42), the signal for the olefinic proton appeared at 6.28 ppm (CCl_4 solution). In the acetate (42a), the signal appeared at 5.91 ppm (CCl_4 solution). The upfield shift of 22.2 cps for this signal in (42a) indicated that the deshielding effect of the phenyl ring on the olefinic proton, which was attributed by Mechoulam and Gaoni to the abnormally low field position of the signal for the olefinic proton in (42), had been decreased in (42a). Such an effect would only be observed if the alicyclic double bond were in the $\Delta^{9,10}$ position, since this is the only position in which the olefinic proton at C−10 is placed. This is the only position in which the olefinic proton is near enough to the phenyl ring to be significantly influenced by the change in the phenyl moiety caused by the acetylation of (42).

Treatment of the acetate (42a) with lithium aluminum hydride at 0°C in diethyl ether yielded the starting material (42). This demonstrated that no isomerization had occurred under the conditions used to acetylate (42).

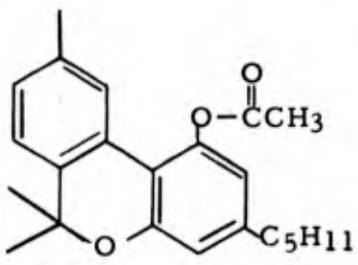
Another example of the effect that acetylation of a 1-hydroxyl group had on a deshielded C−10 proton was obtained from the NMR spectra of cannabinol (1) and cannabinol acetate (1a).

Table X. NMR Spectra of (42) and (42a) in CCl_4

Group	Chemical shift from tetramethylsilane		$\delta_{(42)} - \delta_{(42a)}$
	(42)	(42a)	
	ppm		Δ cps
CH_3 of n-amyl	0.88 (triplet)	0.88 (triplet)	–
CH_3 of acetyl	–	2.18 (sharp singlet)	–
CH_3 at C–9	1.63 (broad doublet, J = 1.0 cps)	1.63 (broad singlet)	–
CH_3's at C–6	1.05, 1.37 (sharp singlets)	1.05, 1.35 (sharp singlets)	–
C–10a proton	3.10 (broad doublet, J = 8 cps)	2.97 (broad doublet, J = 8 cps)	1.8
Olefinic proton	6.28 (broad multiplet) 5.93 (doublet, J = 2 cps)	5.91 (broad multiplet) 6.25 (doublet, J = 2 cps)	22.2 -19.2
Aromatic protons	6.10 (doublet, J = 2 cps)	6.42 (doublet, J = 2 cps)	-19.2



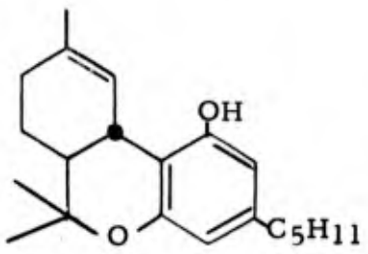
(1)



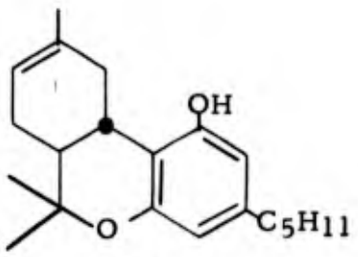
(1a)

The main point of interest in the NMR spectra of (1) and (1a) is the chemical shifts of the C–10 protons. In cannabinol, the C–10 proton appeared at 8.13 ppm. This was 70 cps downfield from the other two aromatic protons (6.98 ppm) of the A ring. A Dreiding model of (1) showed that the two phenyl rings in this molecule lie in the same plane. The additional deshielding of the C–10 proton must arise from the anisotropic deshielding effect of ring C on this proton. That this was an effect through space was illustrated by the NMR spectra of (1a). The C–10 proton in (1a) appeared at 7.67 ppm, 27.6 cps

upfield from its position in (1). The other two aromatic protons of ring A, however, appeared at 6.91 ppm, which was only 4.8 cps upfield from their position in (1). Since upfield shifts of signal for the C−7 and C−8 protons were much smaller than for the C−10 proton, this showed that the larger upfield shift of the C−10 proton in (1a) was not due to an electronic effect through the bonds of (1a). An electronic effect would have caused the upfield shifts in signal positions of the C−7, C−8, and C−10 protons in (1a) to be of about the same magnitude. The structure and conformation of cannabinol (1) have been well established. Since the shielding effect on the C−10 proton caused by acetylation of cannabinol was of the same type and magnitude as the shielding effect on the C−10 proton caused by acetylation of the natural tetrahydrocannabinol (42), this added more support to the structure assigned to the naturally occurring tetrahydrocannabinol.



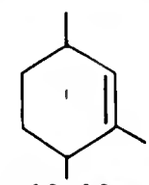
(42)



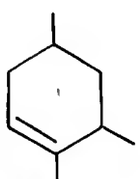
(93)

The other noncrystalline tetrahydrocannabinol, $[\alpha]_D$ -260°, was assigned structure (93). Adams and coworkers[25] had reported the isolation of a tetrahydrocannabinol of the same structure from the cyclization of cannabidiol with p-toluenesulfonic acid in refluxing benzene. The NMR spectrum of (93) is shown in table XI.

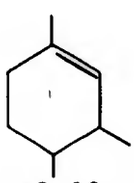
The NMR spectrum of (93) showed the presence of four methyl groups. A distorted triplet at 0.88 ppm was assigned to the methyl of the n-amyl group. The methyls of the pyran ring and the cyclohexene ring of (95) appeared as three singlets at 1.10, 1.37, and 1.70 ppm. A broad singlet for one proton at 5.47 ppm was assigned to the olefinic proton of (93). The presence of only one olefinic proton reduced to four, as shown below, the possible positions of the alicyclic double bond in the tetrahydrocannabinol, $[\alpha]_D$ -260°.



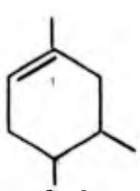
$\Delta^{10,10a}$



$\Delta^{6a,7}$



$\Delta^{9,10}$



$\Delta^{8,9}$

Table XI. NMR Spectrum of (93) in $CDCl_3$

Group	Chemical shift from tetramethylsilane
	ppm
CH_3 of n-amyl	0.88 (triplet)
CH_3 at C−9	1.70 (broad singlet)
CH_3's at C−6	1.10, 1.37 (sharp singlets)
C−10a proton	5.47 (broad singlet)
Olefinic proton	3.25 (broad doublet, J = 15 cps)
Aromatic protons	6.13 (doublet, J = 2 cps) 6.32 (doublet, J = 2 cps)

The $\Delta^{10, 10a}$ position of the alicyclic double bond that is conjugated with the phenyl ring was eliminated on the basis of the UV spectrum, which established that the double bond of (93) was not conjugated with the phenyl ring.

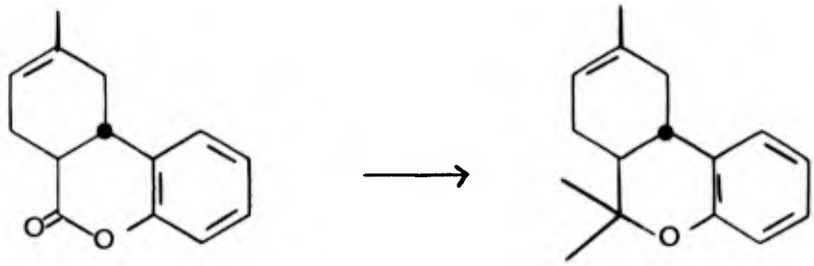
The $\Delta^{6a, 7}$ position of the double bond could be eliminated by comparison of the NMR spectrum of (93) with the NMR spectrum of (44), which had a $\Delta^{6a, 10a}$ position of the alicyclic double bond. The methyl group attached to C−9 in (44) appeared as a doublet (J = 6 cps) at 1.05 ppm. This splitting was attributed to the C−9 methine present in a structure such as (44). The magnitude of the coupling constant was of the order expected for a $-CH-CH_3$ group. A tetrahydrocannabinol with $\Delta^{6a, 7}$ double bond would also contain such a group, and the C−9 methyl would be expected to appear as a doublet with a coupling constant of about 6 cps. Since the methyls other than the methyl of the n-amyl group of (93) appeared as singlets, the methyl group at C−9 in (93) could not be adjacent to a methine proton.

The two remaining positions for the alicyclic double bond in (93) were the $\Delta^{9, 10}$ and the $\Delta^{8, 9}$ positions. The $\Delta^{9, 10}$ position was eliminated on the basis of NMR spectra, IR spectra, and chemical evidence. The optical rotation, NMR spectra, and IR spectra definitely established that the tetrahydrocannabinol that was assigned structure (42) by Gaoni and Mechoulam[47] was not identical to the tetrahydrocannabinol, $[\alpha]_D^{27}$-260°. These two tetrahydrocannabinols could differ from each other in one of two ways. Both could

have the same position of the alicyclic double bond, but differ in the stereochemistry of the ring fusion of the cyclohexene and pyran rings, or both could have the same ring fusion but differ in the positions of the alicyclic double bond. The stereochemistry of the ring fusions in the tetrahydrocannabinols (42) and (93) were shown to be identical by a method first used by Adams and coworkers.[24] Catalytic hydrogenation of (42) yielded an optically active hexahydrocannabinol, $[\alpha]_D^{27}$ -108°. Catalytic hydrogenation of (93) also yielded an optically active hexahydrocannabinol, $[\alpha]_D^{27}$ -109°. The IR spectra of these two materials were identical, which established that (42) and (93) could only differ in the position of the alicyclic double bond. By elimination, the only other possible position for the double bond in (93) was $\Delta^{8,9}$.

More evidence for the assignment of a $\Delta^{8,9}$ position for the double bond of (93) was obtained from a comparison of the position of the olefinic proton signal in the NMR spectra of (42) and (93). The olefinic proton appeared at 6.38 ppm (singlet) in the NMR spectrum of (42). This was an abnormally low field position for an olefinic proton of this type, but, as Gaoni and Mechoulam[47] pointed out, the additional deshielding must arise from the fact that the olefinic proton of (42) is in the plane of the phenyl ring. The signal for the olefinic proton of (93) appeared at 5.42 ppm (broad singlet), which is normal for olefinic protons of this type. This showed that the olefinic proton of (93) must be in a position where no deshielding by the phenyl ring could occur. The only position that satisfies this condition and all the other evidence is $\Delta^{8,9}$.

The NMR spectrum of (93) was also compared to that of a model compound first reported by Strojny and Taylor,[65] in which the position of the alicyclic double bond and the stereochemistry of the ring fusion were known. The model compound, trans-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6-dibenzopyran (60), was prepared by the treatment of trans-9-methyl-6a,7,10,-10a-tetrahydro-6-dibenzopyrone (56) with methylmagnesium iodide to yield an intermediate diol, which, when treated with p-toluenesulfonic acid in refluxing benzene, yielded (60).



(56) (60)

Three signals for methyl groups at 1.10 (sharp singlet), 1.32 (sharp singlet), and 1.68 ppm (broad singlet) were present in the NMR spectrum of (60). A broad singlet for one proton appeared at 5.40 ppm and was assigned to the olefinic proton of (60).

The appearance of the broad signal in the NMR spectrum for the C−9 methyl and C−8 olefinic proton was attributed previously for the pyrones with a $\Delta^{8,9}$ double bond to weak coupling between these groups. The same effect was also present in (60). This broad methyl signal was, by analogy to the pyrones with a $\Delta^{8,9}$ double bond, assigned to the C−9 methyl. The other two methyl signals must then be due to the geminal dimethyl group of (60).

The NMR spectrum of (93) had the same general characteristics as the spectrum of (60). A broad signal for an olefinic proton appeared at 5.47 ppm, compared to 5.40 ppm for (60); and a broad methyl signal for the C−9 methyl appeared at 1.70 ppm in (93) compared to 1.69 ppm for (60). The methyls of the geminal dimethyl group of both (93) and (60) appeared at 1.10 and 1.35 ppm. The close agreement between the NMR spectra of (93) and (60) supported the assignment of the double bond of (93) to the $\Delta^{8,9}$ position.

Gaoni and Mechoulam[47] assigned a trans- configuration to the fusion of the cyclohexene and pyran rings in (42) on the basis of the NMR spectrum. The other evidence presented by Gaoni and Mechoulam for the trans- configuration of the ring fusion in (42) was the mild acid-catalyzed cyclization of cannabidiol to yield (42). These investigators had shown that the phenyl ring and the isopropenyl group of cannabidiol have a trans- configuration by chemical means and by the NMR spectrum of cannabidiol.

Although both the synthetic cis-tetrahydrocannabinol (81) and the natural material (93) have the alicyclic double bond in the $\Delta^{8,9}$ position, their IR and NMR spectra were not identical, thus giving additional support to the assignment of a trans- configuration to (93). Catalytic hydrogenation of (42) and (93) yielded the same hexahydrocannabinol. The IR spectrum of this hexahydrocannabinol is not identical to that of the hexahydrocannabinol obtained by the catalytic hydrogenation of (81). These data established that the ring fusion of the cyclohexene ring and pyran ring of the synthetic tetrahydrocannabinol (81) differed from that of the natural tetrahydrocannabinols (42) and (93). Since a cis- configuration of the ring fusion of the cyclohexene ring and pyran ring in the synthetic tetrahydrocannabinol (81) had been established, the natural tetrahydrocannabinols must have the trans- configuration. The carbon skeletons of these three tetrahydrocannabinols, (81), (42), and (93), were shown to be identical by sulfur dehydrogenation to cannabinol.[1]

The physiological activities of the natural and synthetic tetrahydrocannabinols were tested by intravenous injection in dogs. The dogs used in this experiment had been trained to jump over a barrier when a buzzer was sounded. A control was run on each animal to determine the mean response time for 10 jumps over the barrier. The dogs were injected and tested after 10 min and 2, 4, and 24 hr. The data are summarized in table XII.

Table XII. Physiological Activity of Natural and Synthetic Tetrahydrocannabinols in Dogs

Compound	Dose	Time tested	Response ratio	Mean response time	Temperature before jump/ after jump	Heart rate before jump/ after jump
	mg/kg	hr	n/10	sec	°F	bpm
The mixture	1.0	Control	10	1.49	101.7/101	128/120
of (42) and		1/6	2	6.10	105/105	80/80
(93) present		1	2	2.29	105/105	140/140
in American		2	3	3.39	103/103	160/160
marijuana		4	10	3.44	101/101	132/132
		24	10	1.59	103/103	144/132
(81)	1.0	Control	10	1.65	101/102	110/100
		1/6	10	1.61	102/102	84/80
		1	10	1.87	101/102	80/80
		2	10	1.73	101/101	78/84
		4	10	1.65	101/101	84/84
		24	10	1.64	103/103	120/120
(44)		Control	10	1.53	102/102	124/112
		1/6	10	3.72	100/100	88/88
		1	10	1.92	102/102	100/100
		2	10	2.98	100/100	96/100
		4	10	2.80	101/102	104/100
		24	10	1.73	101/101	124/120
Solid	0.75	Control	10	1.06	102/102	120/120
tetrahydro-		1/6	10	1.39	102/102	84/84
cannabinol,		1	10	1.29	101/101	68/72
mp 86° to		2	10	1.32	102/102	96/92
88°C		4	10	1.38	102/102	100/104
		24	10	1.19	102/102	120/128

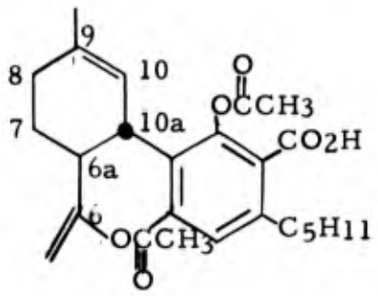
The effects on the behavior of the dog injected with the mixture of the natural tetrahydrocannabinols (42) and (93) were noticed within 10 min and lasted more than 24 hr, even though the dog was able to perform the jump test 4 hr after the administration of the tetrahydrocannabinols. In the instance of the synthetic tetrahydrocannabinol (81), there were no significant changes in the behavior of the dog; however, a large depression in heart rate was noted. This effect was rapid and lasted over 4 hr. The Adams' tetrahydrocannabinol (44) caused the dog to be sleepy and unsteady on his feet 10 min after injection. These effects had disappeared 4 hr after administration. The solid tetrahydrocannabinol, mp 86° to 88°C, which consisted mostly of the material, mp 143° to 145°C, contaminated with small amounts of (42) and (93), showed no significant change in the behavior of the dog.

The investigations on marijuana from various sources showed that the tetrahydrocannabinol content of these materials varies greatly with their origin. A sample of Mexican marijuana contained the two tetrahydrocannabinols (42) and (93). A sample of Spanish marijuana contained only the tetrahydrocannabinol (42). A sample of "red oil" distilled from Egyptian hashish contained mostly the tetrahydrocannabinol (42), but traces of the tetrahydrocannabinol (93) were also detected by TLC. A 3-yr-old sample of marijuana from Mexico yielded only (42) and cannabinol. Samples of West Virginian marijuana contained no tetrahydrocannabinol.

A 1,600-gm sample of West Virginia marijuana harvested in July was extracted at 0°C with 7.5 gal of petroleum ether (30° to 60°C) by percolation through a column packed with the finely pulverized marijuana leaves. The petroleum ether extract was reduced to a volume of 1 ℓ at 25°C on a rotary evaporator, and the extract was washed with a 2% sodium hydroxide solution containing sodium sulfite as an antioxidant.[33] The basic solution was acidified to yield an oil that was extracted with benzene. The benzene solution was dried over magnesium sulfate and then treated with acetic anhydride and sodium acetate for 4 days at room temperature. The benzene solution yielded 12 gm of a red oily substance, which was purified by column chromatography on silica gel to yield 7.3 gm (20% yield based on total resin extracted) of white needles from petroleum ether, mp 124° to 125°C. Recrystallization from hexane yielded a material with two melting points, 95° to 98°C and 124° to 125°C (literature[33,34] mp 80° to 100°C/127° to 128°C and 96° to 97°C/115° to 118°C). Schultz and Haffner[33] attributed the two melting points of this substance to two interchangeable crystalline forms. Santavy and Krejci[34] indicated that the two melting points may be due to the presence of two interconvertible crystalline forms or to two isomers. Attempts to separate the compound that was isolated from the West Virginia marijuana into two components by TLC on silica gel,

silica gel-silver nitrate, magnesium silicate, and alumina were not successful. Countercurrent distribution of the material did not result in any separation. It was found that the solid isolated from the cuts taken from the silica gel column by slow evaporation of the solvent under a stream of nitrogen yielded a solid with only one melting point, 124° to 125°C. Recrystallization of this solid from a hexane that was heated in order to effect solution yielded a solid, mp 96° to 98°C/124° to 125°C. The material with one melting point had the same optical rotation as the material with two melting points, which indicated that the two melting points arise from different crystalline forms. If isomerization had occurred, a change in the optical rotation would be expected.

The optical rotation of the substance isolated from West Virginian marijuana did not agree with the data reported for a similar substance by Santavy and Krejci.[34] These investigators reported $[\alpha]_D$ -114° for the substance isolated from Cannabis sativa grown in Czechoslovakia. The substance isolated from West Virginian marijuana had an optical rotation of $[\alpha]_D$ -60°. A sample of a substance isolated from Cannabis sativa of German origin by Schultz and Haffner[33] was obtained from Professor Schultz. This material had a rotation of $[\alpha]_D$ -57°. The IR spectrum of this substance was identical to the IR spectrum of the substance isolated from West Virginia marijuana. On the basis of these data, the substance from West Virginia marijuana appeared to be cannabidiolic acid diacetate, 3-(2,6-diacetoxy-3-carboxy-4-n-amylphenyl)-4-isopropenyl-1-methylcyclohexene (94).



(94)

The NMR spectrum of (94) is shown in table XIII.

The NMR spectrum of (94) showed the presence of five methyl groups that were the methyl of n-amyl group, the two methyls of the acetoxy groups, and two allylic methyl groups. The presence of two allylic methyl groups indicated that the alicyclic double bond of (94) was either in the $\Delta^{9,10}$ or $\Delta^{8,9}$ position. This assignment was supported by the presence of signals

for only three olefinic protons. On the basis of the chemical shift, the signal at 4.50 and 4.58 ppm was assigned to the terminal methylene group of (94).[73] The signal at 5.23 ppm for the single-ring olefinic proton established that one terminus had to be at C−9. It was shown by catalytic hydrogenation of (94) that the methine proton at C−10a was allylic. Catalytic hydrogenation of (94) yielded a crystalline compound, mp 148° to 149°C. The NMR spectrum of this compound showed that the signal for the C−10a proton was shifted from 3.51 ppm to about 2.80 ppm where it overlapped with the signal from the methylene of the n-amyl group α to the phenyl ring. These data established that the C−10a proton in (94) was deshielded by an adjacent double bond. Reduction of this double bond destroyed the deshielding effect and caused the signal for the C−10a proton to shift upfield. This phenomenon would only occur if the alicyclic double bond of (94) were situated in the $\Delta^{9,10}$ position. These data are in agreement with the data found by Mechoulam and Shvo[26] for cannabidiol, to which they assigned a $\Delta^{9,10}$ double bond.

Table XIII. NMR Spectrum of (94) in $CDCl_3$

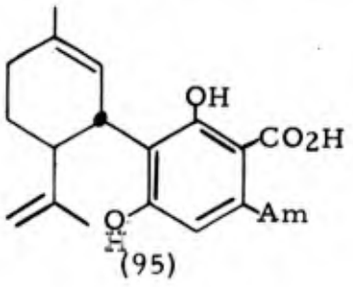
Group	Chemical shift from tetramethylsilane
	ppm
Methyl of n-amyl	0.87 (triplet)
Methyl at C−6	1.58 (sharp singlet)
Methyl at C−9	1.67 (broad singlet)
Methyls of acetoxys	2.20 (sharp singlet)
C−10a proton	3.53 (doublet, J = 12 cps)
Ring olefinic proton	5.23 (broad singlet)
$=CH_2$ group	4.50; 4.58 (broad singlets)
Aromatic proton	6.87 (singlet)

A carboxyl group was shown to be present in (94) by a broad hydroxyl band at 3500 to 2500 cm^{-1} and a carbonyl band at 1700 cm^{-1} in the IR spectrum. The presence of one exchangable proton was also shown by the NMR spectrum. The acetoxy groups of (94) were indicated by the carbonyl band at 1775 cm^{-1} in the IR spectrum and the signal for two methyls at 2.20 ppm in the NMR spectrum.

Additional evidence for the structure of cannabidiolic acid diacetate (94) was obtained by the mild basic hydrolysis of this material. Treatment of (94) under a nitrogen atmosphere at room temperature for 1 day with 2% sodium hydroxide solution yielded two products, which were shown to be cannabidiolic acid (95) and cannabidiol (33) by TLC and paper chromatography. Since the mild conditions used in the hydrolysis of (94) would not be expected to cause any isomerization, these data showed that the stereochemistry at C−6a and C−10a and the position of the alicyclic double bond must be the same in (94) as in cannabidiol (33). Mechoulam and Shvo[26] have shown that cannabidiol (33) has a trans- diequatorial relationship of the isopropenyl group and the phenyl ring, and a $\Delta^{9,10}$ alicyclic double bond.

A second sample of mature West Virginian marijuana was harvested near the end of September from the same field as the material obtained in July. A 945-gm sample of this material was treated as described previously to yield 4.5 gm of cannabidiolic acid diacetate (94). The ratio of the weight of (94) to the total weight of marijuana in this instance was 4.8×10^{-3} as compared to a ratio of 4.5×10^{-3} for the marijuana harvested in July. These data showed that, for this variety of Cannabis, the green plants contained essentially the same amount of cannabidiolic acid as the mature plants. In neither instance could any tetrahydrocannabinol be detected; however, traces of cannabidiol were found in both instances.

An 8-lb sample of West Virginian marijuana that was kept in the dark and at room temperature for 8 mo was extracted in the usual manner with petroleum ether. TLC of the petroleum ether extract showed the presence of cannabidiolic acid, cannabidiol, and a trace amount of the tetrahydrocannabinol (42). The cannabidiolic acid was isolated by a base extraction of the petroleum ether extract. On acidification, the basic extract yielded a yellow oil, which was extracted with ether. Evaporation of the ether solution yielded 14 gm of a tan acidic solid that was dissolved in petroleum ether-ether (9.5:0.5). A fine powder precipitated from this solution on cooling. This solid decomposed with the evolution of carbon dioxide at 120° to 130°C. The IR spectrum of this substance was in agreement with the IR spectrum of cannabidiolic acid published by Schultz and Haffner.[33] The optical rotation of this solid, $[\alpha]_D$ -120°, compares favorably with the optical rotation of $[\alpha]_D$ -115° ±4° reported by Santavy and Krejci[34] for cannabidiolic acid (95).



(95)

The NMR spectrum of (95) showed the presence of an aliphatic methyl group at 0.88 ppm (triplet) and two methyl groups attached to olefinic carbon atoms at 1.65 and 1.89 ppm. This placed the alicyclic double bond in either the $\Delta^{9,10}$ or $\Delta^{8,9}$ positions. The position of the signal for the C−3 proton at 4.08 ppm (broad singlet) established that the alicyclic double bond is in the $\Delta^{9,10}$ position for the reasons previously described for cannabidiolic acid diacetate (94). The protons of the C−6 methylene group appear at 4.45 and 4.55 ppm in (95). The C−2 olefinic proton appears as a broad singlet at 5.58 ppm and the phenyl protons as a sharp singlet at 6.38 ppm.

The stereochemistry of the C−6a and C−10a carbon atoms in (95) was shown to be the same as in cannabidiol. An ethanol solution of (95) was allowed to stand at room temperature for 5 days. TLC of this solution showed that it still contained a small amount of (95) but that the major component was cannabidiol. The ethanol was distilled off and the red, resinous residue dissolved in petroleum ether. The petroleum ether solution yielded a crystalline compound, mp 66° to 68°C, $[\alpha]_D$ -131°, which had an IR spectrum identical to that of an authentic sample of cannabidiol. Since the decarboxylation of (95) occurred under such mild conditions, it can be assumed that no isomerization had occurred,and the position of the double bond and the stereochemistry of the C−10a and C−6a atoms are the same in both cannabidiolic acid (95) and cannabidiol (33).

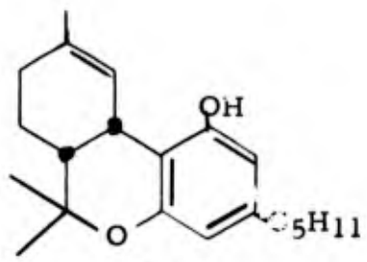
A series of experiments was conducted to determine if the naturally occurring tetrahydrocannabinols could be formed from cannabidiolic acid. Depending on the reaction conditions, one or more of four tetrahydrocannabinols were formed by the acid-catalyzed decarboxylation and cyclization of cannabidiolic acid (95).

Treatment of (95) in refluxing ethanolic hydrogen chloride for 15 hr yielded a mixture of three tetrahydrocannabinols. This mixture was separated into two components by column chromatography on silica gel-silver nitrate. The fastest-moving component eluted from the column had an optical rotation of $[\alpha]_D$ -279° and was shown to be a mixture of two tetrahydrocannabinols by the NMR spectrum. The NMR spectrum of this material showed the presence of six different methyl groups. The aliphatic methyl of the n-amyl group was observed at 0.88 ppm (triplet). Five other methyl signals, all singlets, were observed at 1.09, 1.35, 1.62, 1.68, and 1.92 ppm. Two different olefinic protons are indicated by two peaks in a ratio of 2:1 at 5.45 ppm (broad singlet) and 4.21 ppm (broad singlet). The combined signals at 5.45 and 4.21 ppm accounted for only one proton. The positions of the methyl resonances and the olefinic proton resonances showed that the material,

$[\alpha]_D$ -279°, was composed of 67% trans-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyran (93) and 33% cis-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyran (81).

The second compound isolated from the treatment of (95) with ethanolic hydrogen chloride had an optical rotation of $[\alpha]_D$ -49°. The UV spectrum of this compound had absorption maxima at 282 (ε 1380), 275 (ε 1360), 232 (ε 10, 600), and 208 mμ (ε 41, 600), which indicated a tetrahydrocannabinol with an unconjugated alicyclic double bond.

The NMR and IR spectra showed this tetrahydrocannabinol was different from the three tetrahydrocannabinols of known structure, cis- and trans-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyran (81) and (93) and trans-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 8, 10a-tetrahydro-6-dibenzopyran (42), which have one terminus of the alicyclic double bond on C−9. The only other possible structure for a tetrahydrocannabinol with one terminus of the alicyclic double bond on C−9 is cis-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 8, 10a-tetrahydro-6-dibenzopyran (96).



(96)

The presence of only one olefinic proton and three deshielded methyl groups in the NMR spectrum of (96) indicated that one terminus of the double bond was C−9 (table XIV). This was proved by the NMR spectrum of the catalytically hydrogenated tetrahydrocannabinol. The NMR spectrum of the hydrogenated material showed that no olefinic proton was present. The two methyl groups that appeared at 1.62 and 1.89 ppm in the spectrum of the tetrahydrocannabinol had undergone a shift to a higher field in the hydrogenated product. One methyl was shifted to 1.31 ppm and the other to 1.05 ppm. The methyl at 1.05 ppm appeared as a doublet with a coupling constant of 8 cps. This showed that a methine coupled with the C−9 methyl was present on C−9. For a methine to be present on C−9 in the hydrogenated product, one terminus of the double bond in the unsaturated compound from which the hydrogenated product was formed had to be on C−9. The other methyl, which had shifted

to 1.31 ppm, overlapped with the second methyl on the geminal dimethyl group, and both methyls of this group appeared as a sharp singlet at 1.31 ppm in the hydrogenation product.

Table XIV. NMR Spectrum of (96) in $CDCl_3$

Group	Chemical shift from tetramethylsilane
	ppm
CH_3 of n-amyl	0.88 (triplet)
CH_3's at C−6 CH_3 at C−9	1.33, 1.88, 1.71 (singlets)
C−10a proton	3.50 (broad multiplet)
Olefinic proton	4.97 (broad singlet)
Aromatic protons	6.13 (doublet, J = 2 cps) 6.30 (doublet, J = 2 cps)

The position of the signal for a shielded olefinic proton at 4.97 ppm in the NMR spectrum supported the assignment of a cis- configuration to the fusion cyclohexene and pyran rings in (96). In the instance of (42), which has the same position of the double bond as (96) but a trans- configuration of the ring fusion, the signal for the olefinic proton appeared at 6.37 ppm. The abnormally low field position for this signal was due to deshielding of the olefinic proton by the phenyl ring. The phenyl ring lies in the same plane as the olefinic proton. In (96), Dreiding models showed that the olefinic proton was not held rigidly in the plane of the phenyl ring, but was free to move above and below the plane of the phenyl ring. In the conformations in which the olefinic proton is above or below the plane of the phenyl ring, the olefinic proton would be shielded. This accounted for the high field position of the signal for the olefinic proton in (96) and showed that (96) must have a cis- configuration of the ring fusion.

Supporting evidence for the assignment of structure (96) to the tetrahydrocannabinol $[\alpha]_D$ -49° was obtained from TLC. TLC of the synthetic and natural tetrahydrocannabinols on silica gel-silver nitrate (5:1) showed this system only differentiated between tetrahydrocannabinols that have different

positions of the alicyclic double bond. Tetrahydrocannabinols that have the same position of the alicyclic double bond, but different configuration of the ring fusion of cyclohexene and pyran rings, have the same R_f values using this system. The tetrahydrocannabinol (96), the naturally occurring tetrahydrocannabinols (42) and (93), and the synthetic tetrahydrocannabinol (81) were chromatographed on the same silica gel-silver nitrate plate using benzene as the developer. The tetrahydrocannabinols (81) and (93), which have the double bond in the $\Delta^{8,9}$ position, have the same R_f value. The tetrahydrocannabinols (42) and (96) also have the same R_f values. Since (42) was shown to have a $\Delta^{9,10}$ position for the alicyclic double bond, it seems likely on this basis that (96) also has a $\Delta^{9,10}$ position for the alicyclic double bond.

Catalytic hydrogenation of (96) yielded a hexahydrocannabinol that had an IR spectrum that was very similar to the IR spectrum of the hexahydrocannabinol obtained from the hydrogenation of cis-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyran (81). This supported the assignment of a cis- ring fusion to the cyclohexene and pyran rings of (96).

Treatment of cannabidiolic acid with 85% phosphoric acid in refluxing ethanol yielded a product, $[\alpha]_D$ -130°. The NMR spectrum showed that this material was a mixture of 70% trans-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 8, 10a-tetrahydro-6-dibenzopyran (42) and 30% cis-1-hydroxy-3-n-amyl-6,-6, 9-trimethyl-6a, 7, 8, 10a-tetrahydro-6-dibenzopyran (96). Adams and coworkers[25] reported a rotation of $[\alpha]_D$ -130° for a compound they obtained from the treatment of cannabidiol (33) with ethanolic hydrogen chloride. They concluded that this compound was a homogeneous material, since the experiment was repeated several times and a compound of the same rotation was isolated each time.

Treatment of cannabidiol with p-toluenesulfonic acid in refluxing xylene yielded a compound with an optical rotation of $[\alpha]_D$ -229°. The NMR and IR spectra of this material were identical to the NMR and IR spectra of trans-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyran (93). Treatment of (95) with p-toluenesulfonic acid in refluxing toluene or benzene yielded a compound, $[\alpha]_D$ -261°. The NMR and IR spectra of this compound were identical to the IR spectrum of (93). The same compound, $[\alpha]_D$ -260°, was obtained by refluxing (95) with hydrochloric acid in aqueous acetone. The difference in rotations obtained on treatment of cannabidiol and cannabidiolic acid with p-toluenesulfonic acid cannot be explained, since the spectral data for the two sets of products are identical.

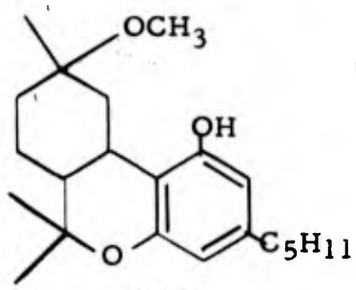
Treatment of a methanol solution of (95) with hydrochloric acid yielded a mixture of (42), (93), and starting material.

The treatment of a methanol solution of (95) with hydrochloric acid at room temperature for 2 wk yielded mostly a methoxy derivative (97) of tetrahydrocannabinol, $[\alpha]_D$-41°, mp 92° to 93°C, and small amounts of (93) and (96).

The NMR spectrum of (97) was in many respects similar to the NMR spectra of the previously described tetrahydrocannabinols (table XV). Two major differences were apparent, however. First, no signal for an olefinic proton was observed, which indicated that if (97) had an alicyclic double bond, it must be in the $\Delta^{6a,10a}$ position where no olefinic proton would be present. This was shown not to be true by the UV spectrum, which established that a $\Delta^{6a,10a}$ alicyclic double bond conjugated with phenyl ring was not present in (97). The second difference in the NMR spectrum of (97) was a sharp singlet at 3.40 ppm for three protons, which established the presence of a methoxyl group in (97). The chemical shift of the methoxyl group indicated that it must be on a saturated carbon.[73] The methoxyl group was placed at C−9 on the basis of the signal observed for the methyl at C−9 in the NMR spectrum of (97). The C−9 methyl was one of three sharp singlets for deshielded methyl groups at 1.15, 1.23, and 1.45 ppm. The other two signals were due to the two methyl groups at C−6. Since no alicyclic double bond was present in (97), the deshielding of the C−9 methyl group had to arise from the oxygen atom of the methoxyl group. Such an effect would have been observed only if the methoxyl group were on C−9.

Table XV. NMR Spectrum of (97) in $CDCl_3$

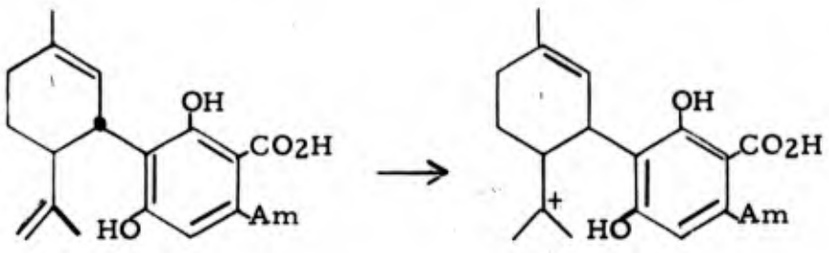
Group	Chemical shift from tetramethylsilane
	ppm
CH_3 of n-amyl	0.88 (triplet)
CH_3's at C−6 CH_3 at C−9	1.15, 1.20, 1.43 (sharp singlets)
$-OCH_3$	3.40 (sharp singlet)
C−10a proton	3.13 (multiplet)
Aromatic protons	6.20 (doublet, J = 2 cps) 6.31 (doublet, J = 2 cps)

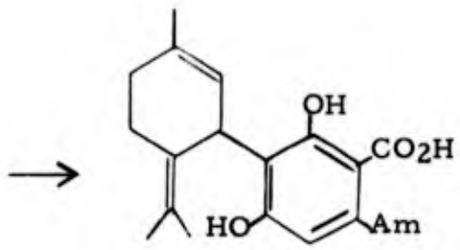


(97)

The presence of the pyran ring in (97) was indicated by the presence of two nonequivalent phenyl protons at 6.21 (doublet, J = 2 cps) and 6.32 ppm (doublet, J = 2 cps). The NMR spectra of all the natural and synthetic tetrahydrocannabinols that have the pyran ring showed that the phenyl protons were not equivalent and were coupled with each other with a coupling constant of 2 cps. In compounds such as cannabidiol, which does not have the pyran ring, the protons are equivalent and show only one sharp peak in the NMR spectrum. On the basis of the NMR spectrum and elemental analysis, the methoxy derivative of tetrahydrocannabinol is 1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-9-methoxy-6a, 7, 8, 9, 10, 10a-hexahydro-6-dibenzopyran (97). No data were obtained for the stereochemistry of the ring fusion in (97).

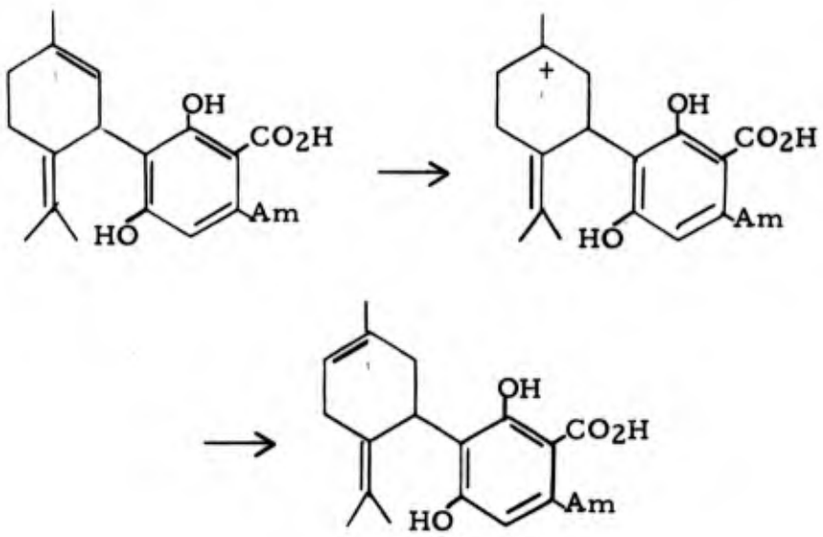
The data obtained from the acid-catalyzed cyclization and decarboxylation of cannabidiolic acid (95) show that it could serve as the precursor for the formation of the tetrahydrocannabinols in the hemp plant. These experiments also show that the two tetrahydrocannabinols found in some samples of marijuana could arise from a single precursor. The appearance of both cis- and trans-tetrahydrocannabinols from the cyclization of cannabidiolic acid (95), which has a trans- configuration of the isopropenyl and phenyl groups, can be explained by the fact that the isopropenyl group can rearrange readily to the isopropylidene group.





(98)

Cyclization and decarboxylation of (98) would give a mixture of cis- and trans-tetrahydrocannabinols with a $\Delta^{9,10}$ position of the alicyclic double bond. The cis- and trans-tetrahydrocannabinols with a $\Delta^{8,9}$ position of the alicyclic double bond could arise by rearrangement of the alicyclic double bond in (98) to form (99).



(99)

Cyclization and decarboxylation of (99) would yield the cis- and trans-tetrahydrocannabinols with the alicyclic double bond in the $\Delta^{8,9}$ position.

The acid-catalyzed cyclization and decarboxylation experiments on cannabidiolic acid demonstrated that the type of isomerization that occurred depends on the acid catalyst used. Hydrochloric acid caused both isomerization of the alicyclic double bond and the ring fusion. p-Toluenesulfonic acid resulted only in the isomerization of the alicyclic double bond. Phosphoric acid caused only isomerization of the ring fusion. Treatment of the trans-tetrahydrocannabinol with the $\Delta^{9,10}$ position of the alicyclic double bond in

refluxing benzene resulted in isomerization of the double bond to the $\Delta^{8,9}$ position, as was reported by Adams.[25] The rearrangement of the alicyclic double bond in (42) also occurred slowly at room temperature in the absence of a catalyst. Irradiation of a hexane solution of (94) with UV light resulted in extensive degradation and some isomerization. One of the products formed by this treatment was shown by TLC to be the $\Delta^{8,9}$-tetrahydrocannabinol (93). Treatment of (42) with p-toluenesulfonic acid in toluene at 100°C for 10 hr, resulted in over 90% conversion, as shown by the NMR spectrum, to the $\Delta^{8,9}$ tetrahydrocannabinol (93).

VII. TLC OF TETRAHYDROCANNABINOL.

The TLC system that proved to be the most useful for the separation of the double-bond isomers of the tetrahydrocannabinols and the other phenolic components found in Cannabis extracts was a weight-to-weight ratio of five parts of silica gel to one part of silver nitrate. The plates were prepared by dissolving the silver nitrate in the water used for making the silica gel slurry. The silica gel slurry was spread on 5- by 20- or 20- by 20-cm glass plates in a 250μ-thick layer. The plates were air-dried overnight. About 3 to 4 ml of a 1% solution in ether of the materials to be chromatographed were spotted 2 cm from the bottom of the plate with a melting-point capillary that had been drawn out into a fine tip. The plates were developed with benzene. The solvent front was allowed to move 10 cm above the point of application. The plates were air-dried for 15 min and then sprayed with a 2% solution of Fast Blue Salt B (E. Merck). This reagent gave bright-red to violet colors with the various phenolic components present in Cannabis extracts. The following figure illustrates a typical chromatogram.

VIII. SUMMARY AND CONCLUSIONS.

The composition of the phenolic constituents of marijuana varies widely with the origin of the samples. While four of the five samples contained one or two isomeric tetrahydrocannabinols and cannabinol or cannabidiol, or both, the fifth specimen yielded only cannabidiolic acid. The cyclization of cannabidiolic acid proceeds with simultaneous elimination of carbon dioxide to yield four isomeric tetrahydrocannabinols. Two of these were found to be identical to the two isomeric, natural trans-tetrahydrocannabinols present in some marijuana specimens. The other two isomers obtained by the partial synthesis from cannabidiolic acid had cis- configuration and were optically active as were the trans- isomers. The attempted total synthesis of a tetrahydrocannabinol identical to one of the natural isomers demonstrated that the approach involving a Diels-Alder condensation was totally unsuitable since this reaction yielded cis- adducts and since the unnatural cis-tetrahydrocannabinols could not be isomerized to the natural trans- compounds.

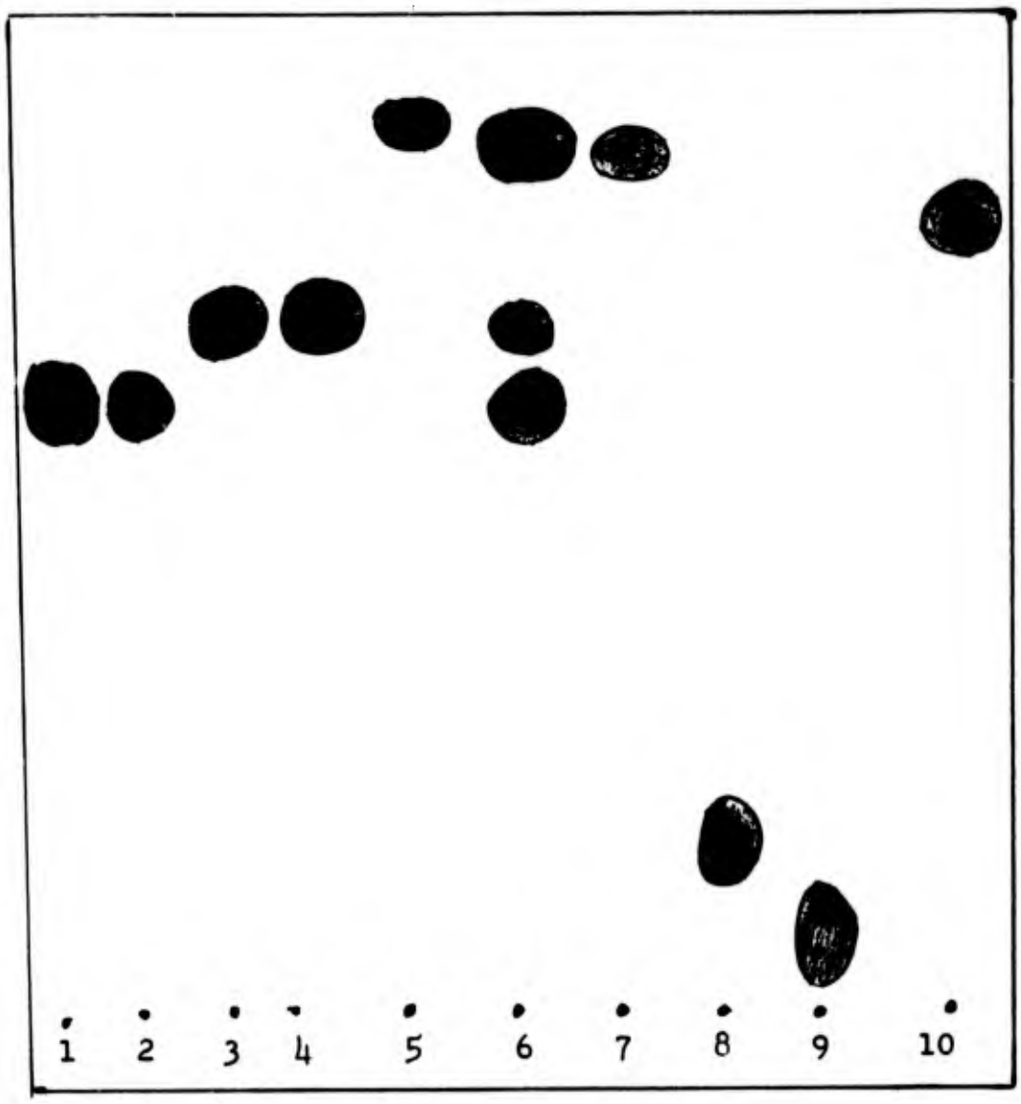


Figure. Thin-Layer Chromatogram of Phenolic Constituents of Cannabis and Synthetic Tetrahydrocannabinols on Silica Gel-Silver Nitrate(5:1), Using a Benzene Developer

1, (96); 2, (42); 3, (93); 4, (81); 5, (44); 6, Solid tetrahydrocannabinol from marijuana, mp 86° to 88°C; 7, solid tetrahydrocannabinol from marijuana, mp 143° to 145°C; 8, (33); 9, cannabidiolic acid; 10, (1)

In addition to cannabinol, cannabidiol, and trans-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 8, 10a-tetrahydro-6-dibenzopyran (tetrahydrocannabinol A), a new marijuana constituent, trans-1-hydroxy-3-n-amyl-6,6,9-trimethyl-6a, 7, 10, 10a-tetrahydro-6-dibenzopyran (tetrahydrocannabinol B), was isolated from Maryland and Mexican marijuana. Traces of tetrahydrocannabinol B were also found in Egyptian hashish. West Virginia marijuana contained only cannabidiolic acid. A second sample of Mexican marijuana furnished only tetrahydrocannabinol A and cannabinol, while a Spanish sample contained an additional amount of cannabidiol. The structure of tetrahydrocannabinol B was elucidated by chemical and spectral evidence. The partial syntheses of four isomeric tetrahydrocannabinols (A, B, and their cis-isomers) and the total synthesis of the racemic cis-isomer of tetrahydrocannabinol B are also described.

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13. ABSTRACT

In addition to cannabinol, cannabidiol, and trans-1-hydroxy-3-n-amyl-6, 6, 9 trimethyl-6a, 7, 8, 10a-tetrahydro-6-dibenzopyran (tetrahydrocannabinol A), a new marijuana constituent, trans-1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro 6-dibenzopyran (tetrahydrocannabinol B), was isolated from Maryland and Mexican marijuana. Traces of tetrahydrocannabinol B were also found in Egyptian hashish. West Virginia marijuana contained only cannabidiolic acid. A second sample of Mexican marijuana furnished only tetrahydrocannabinol A and cannabinol, while a Spanish sample contained an additional amount of cannabidiol. The structure of tetrahydrocannabinol B was elucidated by chemical and spectral evidence. The partial syntheses of four isomeric tetrahydrocannabinols (A, B, and their cis-isomers) and the total synthesis of the racemic cis-isomer of tetrahydrocannabinol B are also described.

14. KEYWORDS

Synthesis	Tetrahydrocannabinol B	Marijuana
Isolation	Constituent	Hashish
Tetrahydrocannabinol	Cannabidiolic acid	Cannabinol
Tetrahydrocannabinol A	Cannabidiol	

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