Chapter Two

RECENT ADVANCES IN THE CHEMISTRY AND METABOLISM OF THE CANNABINOIDS

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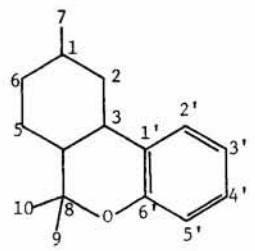
INTRODUCTION

The psychotomimetic activity of the variety of hemp known as *Cannabis sativa* has been known since antiquity. Various preparations of this plant under the names of marijuana, hashish, charas, dagga, and bhang are smoked or chewed by possibly two to three hundred million people. Accordingly, these materials undoubtedly constitute the most widely used group of illicit drugs. The chemistry of these *Cannabis* constituents studied extensively during the 1940's by Todd and his colleagues and the late Roger Adams and his students has been reviewed[1-2-3-4]. However, real progress in the isolation, structural elucidation and synthesis of the various cannabinoids has come only in the last decade, aided by the numerous modern separation methods, analytical techniques, and, most notably, by the development of NMR and mass spectrometry.

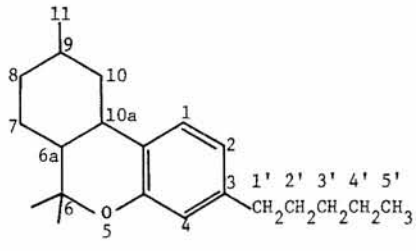
Our knowledge of the metabolism of the cannabinoids is even more recent[1-5], and took place only after the synthetic methodology was placed on a firm basis. In turn, as the knowledge of cannabinoid metabolites has increased, new targets have been given to the synthetic chemist, many of which have already been successfully attained. As a result, pharmacologists, clinicians, and biochemists now have at their disposal a large number of pure cannabinoids, unlabeled, radiolabeled or heavy isotope-labeled, and the number is increasing rapidly. This review will attempt to present the somewhat synergistic manner in which chemistry and metabolism have been interwoven to contribute to recent progress. Of necessity, many of the studies cited will be from the author's point of view and are taken from the work of his laboratory.

NOMENCLATURE OF CANNABINOIDS

There are at least four different numbering systems which have been used in publications relating to cannabinoids. Two of the most common are shown in Figure 1. According to these, the major psychotomimetically active constituent of marijuana would be called Δ^9- or Δ^1-tetrahydrocannabinol (Δ^9- or Δ^1-THC). The Δ^9- nomenclature is utilized in the system for numbering dibenzopyrane compounds and will be used throughout this review. This system is utilized by "Chemical Abstracts" and was adopted by the U.S. National Institute of Mental Health (now National Institute on Drug Abuse). The Δ^1-system was introduced by Mechoulam and has certain general advantages when applied to a large variety of cannabinoids, some of which resemble monoterpenoids



Monoterpenoid



Dibenzopyran

Figure 1

rather than dibenzopyranes. The dual nomenclature for other compounds which will be discussed extensively in this review are 11-hydroxy-Δ^9-THC (7-hydroxy-Δ^1-THC) and 11-nor-Δ^9-THC-9-carboxylic acid (7-nor-Δ^1-THC-1-carboxylic acid). For the latter compound we will use the incorrect but convenient term 11-carboxy-Δ^9-THC.

The structure of Δ^9-THC was established by spectroscopic measurements and chemical correlations[6,7]. Its detailed conformational analysis has been reported[8], and indicates that the cyclohexene ring A in the formula shown in Figure 2 exists in the half chair conformation. As a consequence, substituents at positions 7 and 8 can be regarded as axial or equatorial. In this review hydroxylated constituents are named according to the steroid system. Thus, 7α- and 8β-hydroxy are axial; 7β- or 8α-hydroxy are equatorial constituents.

GLC ANALYSIS OF NATURALLY OCCURRING CONSTITUENTS OF MARIJUANA

Figure 3 shows a number of the naturally occurring constituents which are found in marijuana. Of these, Δ^9-THC, cannabidiol and cannabinol are the most important. Δ^8-THC is probably not found *per se* in plant materials but is an artifact which is produced, in part, upon

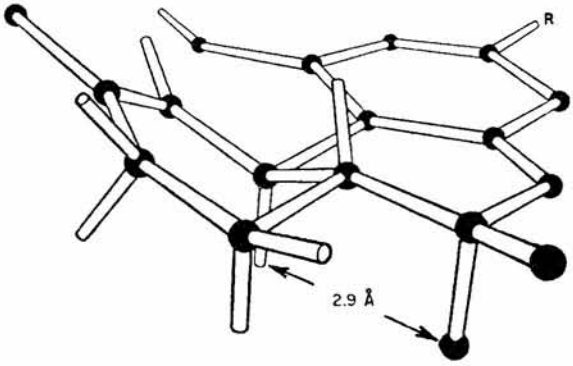
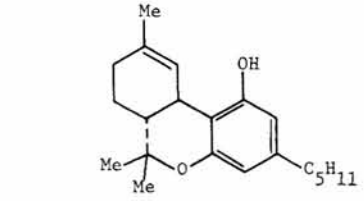
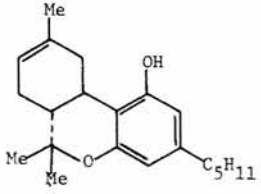


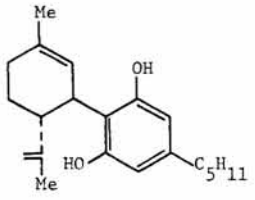
Figure 2. Conformation of Δ^9-THC. (Reference 8. Reprinted with permission of the authors and the Journal of the American Chemical Society.)



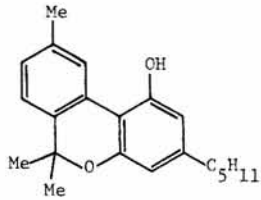
Δ^9-THC



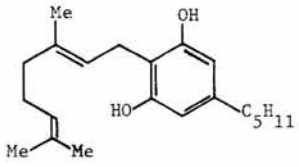
Δ^8-THC



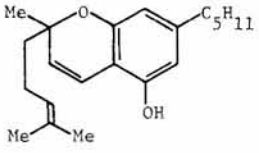
Cannabidiol



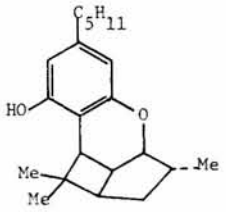
Cannabinol



Cannabigerol



Cannabichromene



Cannabicyclol

Figure 3

pyrolysis, since it is considerably more stable than the Δ^9-analog. Many of the more terpenoid-like cannabinoids were isolated and their structures elucidated by Mechoulam and Gaoni and have been well described in the review by Mechoulam[1]. Another point which should be noted is that many of these constituents, and this is certainly the case of the major ones, occur naturally as the *o*- or *p*-carboxylic derivatives. These carboxylic acid derivatives are quite unstable to heat, and will slowly decarboxylate at room temperature. However, they can be differentiated by gas-liquid chromatography[9]. (The methodology is illustrated in

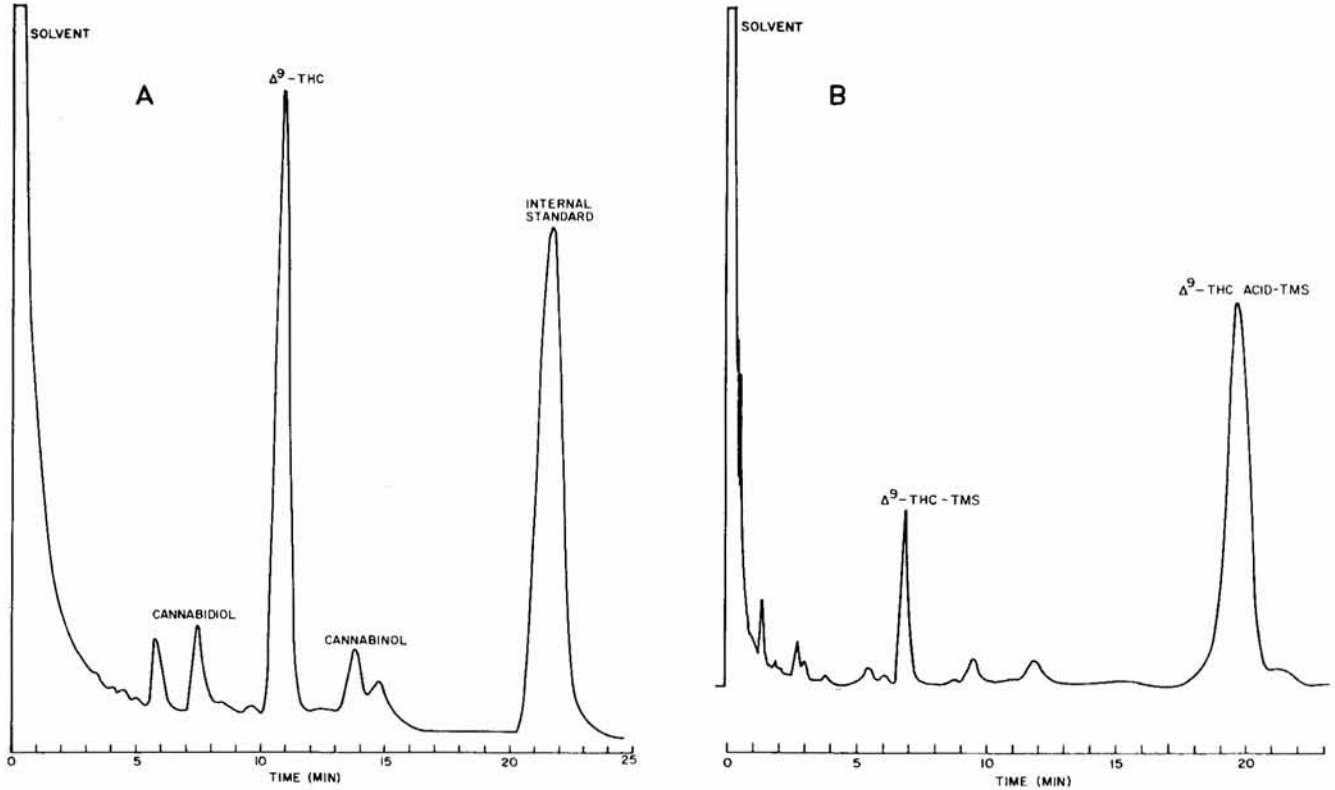


Figure 4. A. Typical GLC of Mexican marijuana extract. B. GLC of derivatized Mexican marijuana extract showing differentiation of Δ^9-THC and Δ^9-THC acid.

Figure 4.) As can be seen, glc of the underivatized marijuana plant extracts shows no signs of any carboxylic acid constituent. However, if the total cannabinoids are converted to the corresponding trimethylsilyl (tms) derivatives, the corresponding carboxylic acid is now stable and can be readily analyzed by glc as a major constituent. From a practical view, however, the underivatized carboxylic THC analogs are so unstable to heat that, when smoked in a normal way by man, the compounds are quantitatively converted to the decarboxylated compounds. The development of accurate glc procedures has permitted facile assessment of the composition of various types of cannabinoid preparations.

There seem to be two major types of *Cannabis* varieties. One form which is exemplified by the type found in Mexico has Δ^9-THC as the predominant constituent. On the other hand, *Cannabis* obtained from Turkey and the near East in general usually has cannabidiol as the major constituent. As another point of interest, compounds analogous to Δ^9-THC, cannabinol, and cannabidiol but with a propyl instead of amyl sidechain have recently been found in samples of Pakistani and Nepalese hashish[10]. In the Nepalese hashish this was a major constituent. Propyl-Δ^9-THC is also biologically active.

SYNTHESIS OF CANNABINOIDS

Following the excellent review on synthetic methods by Mechoulam[1], in this section we will review only methodology of particular importance to the theme of the review. Although various syntheses had been reported previously, the major breakthroughs in this area came in 1967, with the condensation of a pinene derivative, (-)-verbenol, with olivetol in the presence of *p*-toluenesulfonic acid to give an intermediate which could then be further converted to Δ^8-THC as shown in Figure 5.[4] By addition of the elements of hydrochloric acid across the double bond, followed by dehydrochlorination, Δ^9-THC could be produced. Almost simultaneously Petrzilka and coworkers published a method[11,12] which, with modifications, has become the basis for most of the current synthesis of both unlabeled and radiolabeled Δ^9-THC. This method is shown in Figure 6 in a slightly improved and modified form[13] suitable for the synthesis of tritium-labeled Δ^9-THC. The procedure utilizes the readily

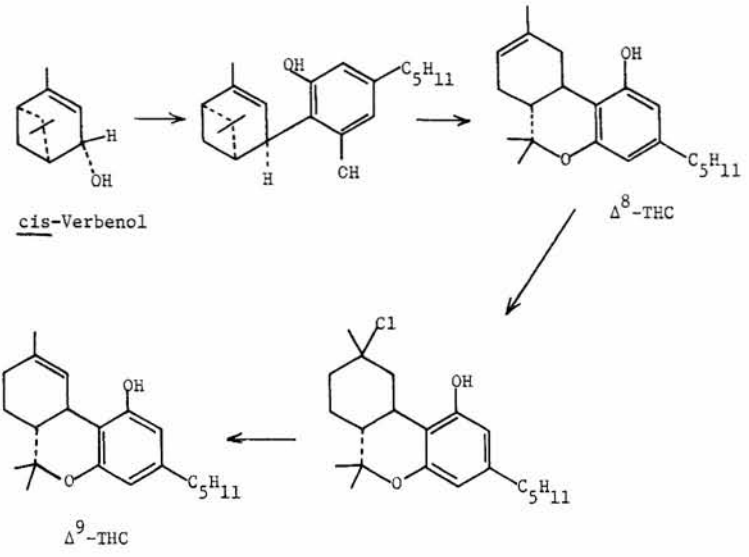


Figure 5

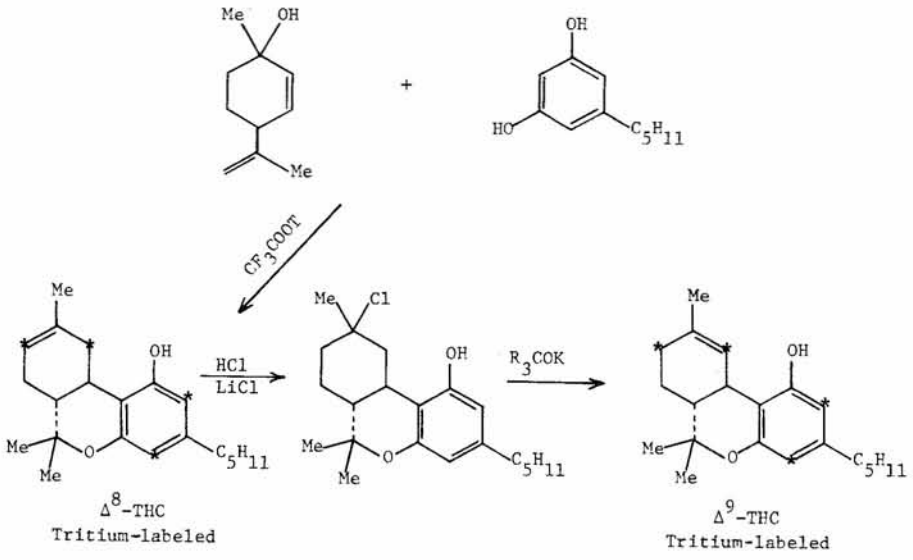


Figure 6

available mentha-2,8-diene-1-ol. In the presence of weak acids, cannabidiol can be obtained directly from this reaction, but with strong acids, such as *p*-toluenesulfonic or trifluoroacetic acid, Δ^8-THC is obtained. These compounds are never obtained pure but usually can be purified by chromatography on adsorbents such as florosil or by high pressure liquid chromatography. Normally the purified Δ^8-THC can be treated in the original Petrzilka method with HCl and then dehydrochlorinated with potassium *tert*-amylate. Although Petrzilka claimed that the final reaction proceeds in very high yield, a number of impurities have been shown to be present by glc. These require a careful and laborious chromatography. This procedure with some modifications can be used to synthesize Δ^9-THC on a kilogram scale.

A more recent procedure in which olivetol is condensed with mentha-2,8-dien-1-ol in the presence of boron trifluoride and magnesium sulfate gives Δ^9-THC directly[14]. Although there are other by-products, the crude Δ^9-THC can be readily purified on a small scale. If the procedure is applicable to a large scale, it may well replace the previously published methodology.

These procedures are not applicable to the preparation of tritium-labeled cannabinoids of high specific activity. For certain purposes, particularly studies involving possible receptor sites, it is absolutely required that the radiolabeled compound be *carrier free*. Under these circumstances, methods of a different nature are required, in which the label is in the sidechain. Gill and Jones published one of the earliest of these procedures in which the tritium label was located in the 1',2'-position of the sidechain, followed by standard condensation of this compound to give radiolabeled Δ^8-THC of rather high specific activity[15]. The method is shown in Figure 7. There are some drawbacks to the procedure. The location of the tritium in the 1'-position places the radiolabel on a benzylic carbon which means that it may be readily exchangeable. Moreover, the highly radioactive intermediate must go through more steps prior to conversion to Δ^8- or Δ^9-THC. Pitt and coworkers have described a method by which a 4',5'-unsaturated analog of olivetol is prepared[16,17] (see Figure 8). The unsaturated olivetol analog is converted in the usual manner to the corresponding Δ^8- or Δ^9-4',5'-unsaturated analog. Radioactivity is introduced by tritiation over a homogenous rhodium catalyst. The procedure produces carrier free Δ^8- or Δ^9-THC. The

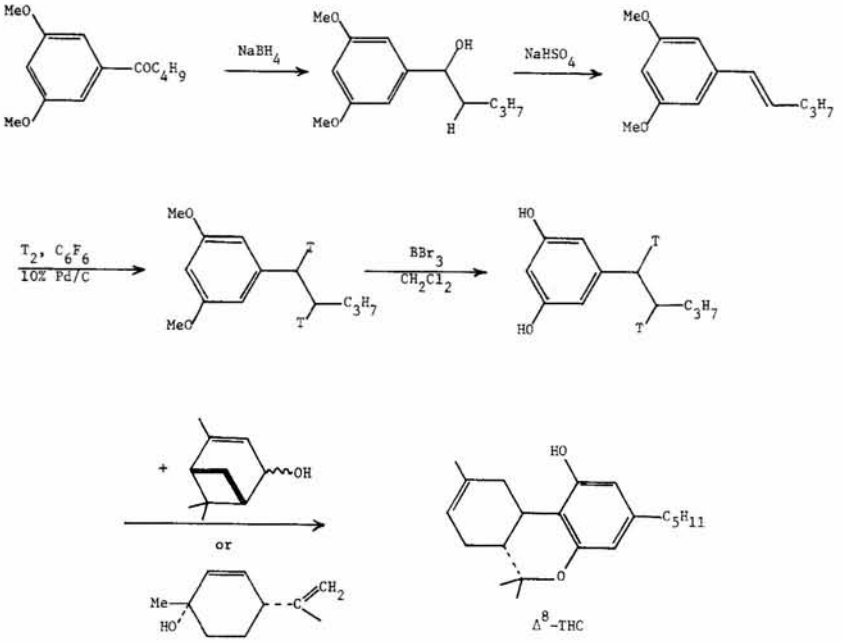


Figure 7

latter compound is unstable and must be used soon after preparation.

In some cases it may be desirable or necessary to use ^{14}C-labeled THC for certain studies, particularly if a metabolic site is involved. We have developed a procedure, outlined in Figure 9, for the synthesis of ^{14}C-Δ^9-THC, labeled at carbon-11. Other methods utilize the synthesis of ^{14}C-labeled olivetol, followed by the conversion of this compound to Δ^8- or Δ^9-^{14}C-labeled THC via condensation with the menthadienol in the standard manner. Our procedure involves conversion of Δ^8-THC to the corresponding $\Delta^{9(11)}$-THC benzyl ether, which is oxidized to give the 9-ketone, and converted to the ^{14}C-labeled $\Delta^{9(11)}$-THC benzyl ether by reaction with the labeled Wittig reagent. Halogenation followed by dehydrohalogenation in the standard manner gives ^{14}C-labeled-Δ^9-THC.

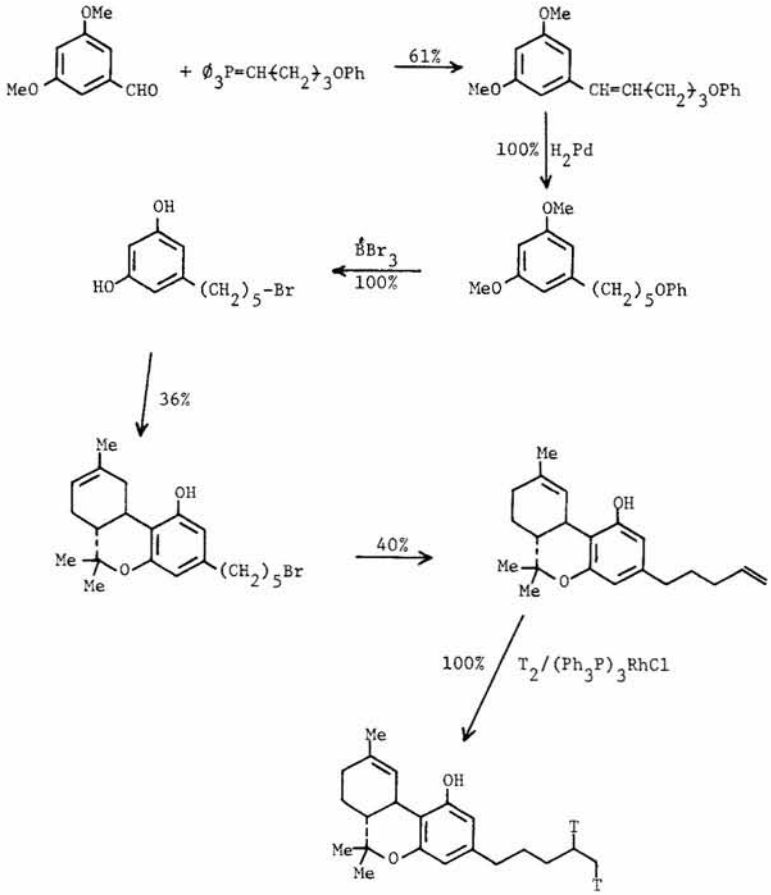

OMe
MeO
CHO
+ Ø3P=CH(CH2)3OPh
61%
CH=CH(CH2)3OPh
100% H2Pd
(CH2)5OPh
BBr3
100%
OH
HO
(CH2)5-Br
36%
Me
Me
O
(CH2)5Br
40%
100% T2/(Ph3P)3RhCl
T

Figure 8

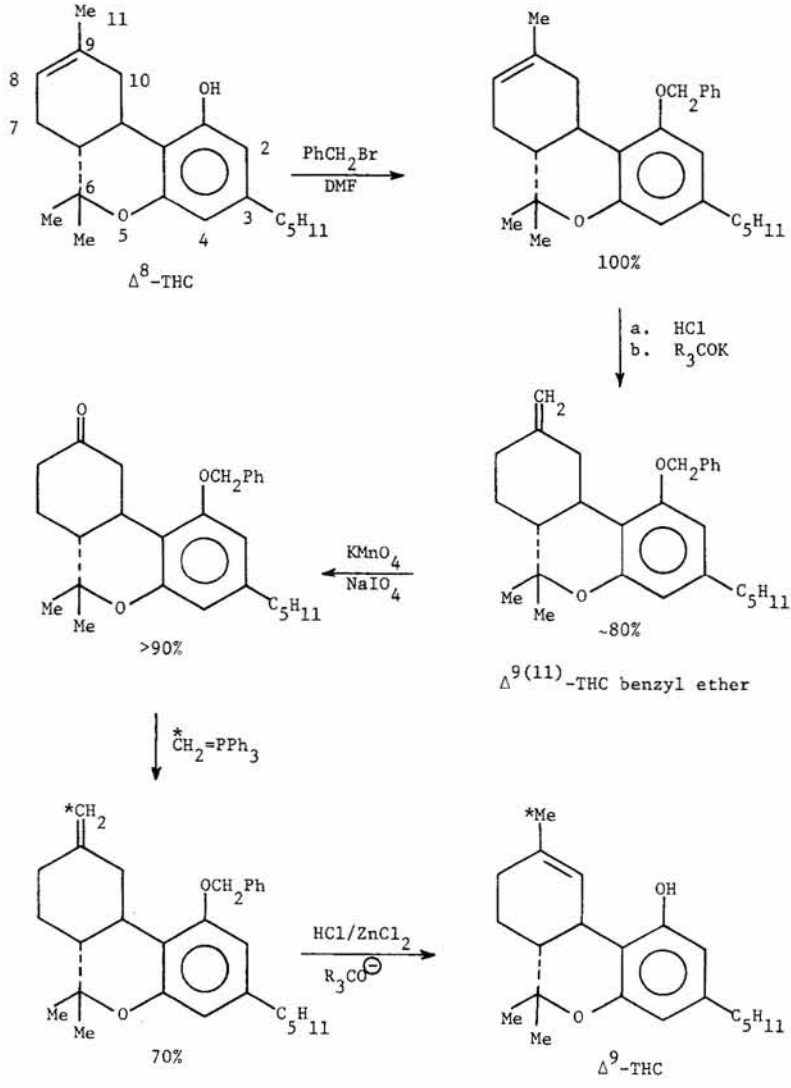

Me
11
9
8
10
OH
7
2
PhCH2Br
DMF
6
Me
O
3
C5H11
Me
5
4
Δ8-THC
OCH2Ph
100%
a. HCl
b. R3COK
CH2
>90%
KMnO4
NaIO4
~80%
Δ9(11)-THC benzyl ether
*CH2=PPh3
*CH2
*Me
HCl/ZnCl2
R3C⊖
70%
Δ9-THC

Figure 9

SYNTHESIS OF METABOLITES

In recent years the metabolism of Δ^9-THC and related compounds has received intensive attention. Many new compounds belonging to this series have been found in biological materials. The synthesis of these metabolites so that their physiological activity and/or toxicity can be determined has become an important challenge to chemists. The work of our laboratory in a few of these areas will be presented below. The synthesis of 11-hydroxy-Δ^9-THC has been an important target, as this compound has high biological activity. As will be shown subsequently, it is a key compound in the metabolism of Δ^9-THC. In 1972, we presented a method[19] which yields not only 11-hydroxy-Δ^9-THC but also the 8α- and 8β-hydroxy-Δ^9-THC (see Figure 10). The latter compounds are also metabolites of Δ^9-THC. Although the yields obtained by this procedure are low, for a long time, it was the only method by which both the "cold" and radiolabeled compounds could be obtained. The method depends on allylic chlorination at the 11- or 8-positions of Δ^9-THC with thionyl chloride followed by formation of corresponding acetates and hydrolysis of the latter. Fortunately, all of the compounds can be separated by careful chromatography. An improved procedure[16,17] which yields 11-hydroxy-Δ^9-THC in 35% yield is shown in Figure 11. Although this procedure involves the formation of several by-products, most of these can be carried along and are minor until the terminal stages of the reaction. At that time the impurities can be readily purified by chromatography. The overall yield of 35% makes this currently the method of choice for the synthesis of either unlabeled or radiolabeled Δ^9-11-hydroxy-THC. A procedure described by Razdan and coworkers[19] utilizes as a precursor the exo-methylene, as shown in Figure 12. This precursor is relatively unavailable to most workers, but it can, if necessary, be made by total synthesis. In any case, as indicated, the compound can be converted into a mixture of Δ^8- and Δ^9-11-acetoxy-THC. After hydrolysis the mixture can be separated by high pressure liquid chromatography. Razdan claims that the procedure has preparative value.

There is also considerable interest in 11-nor-Δ^8- or Δ^9-THC-9-carboxylic acid (11-carboxy-Δ^9-THC). A synthesis for the Δ^8- analog is shown in Figure 13. This method[18] can also be used for the facile synthesis of 11-hydroxy-Δ^8-THC. In the case of the Δ^9- series, the synthesis of

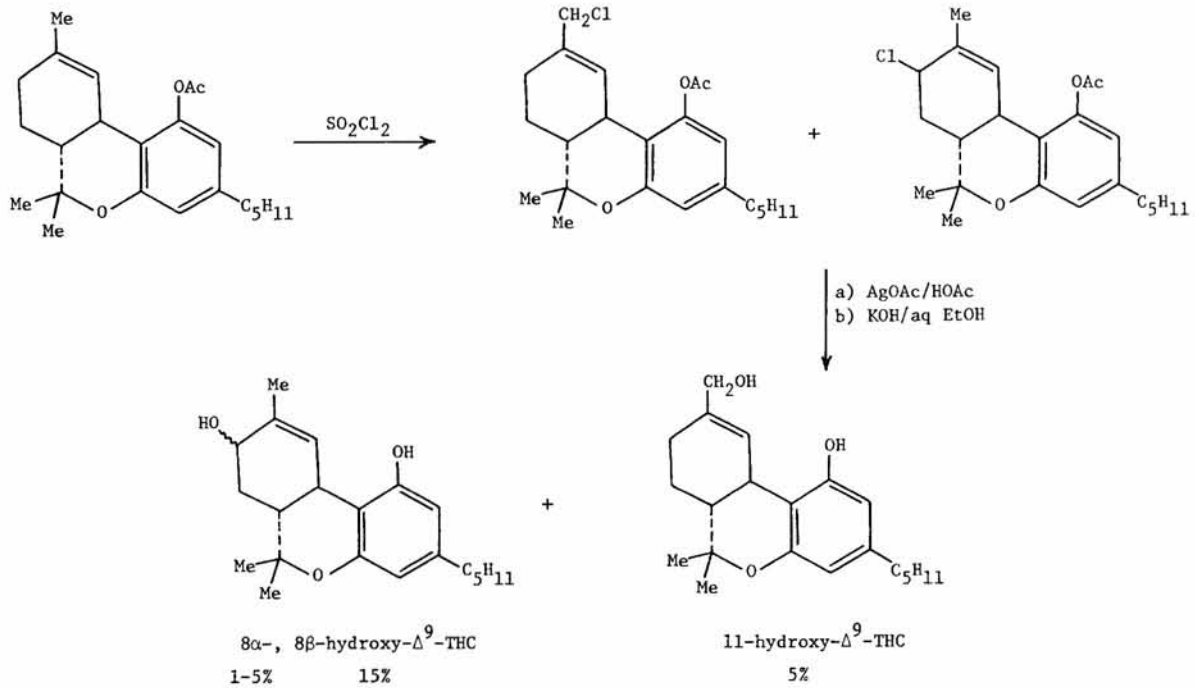

Me
OAc
SO_2Cl_2
CH_2Cl
Cl
C_5H_{11}
Me
O
a) AgOAc/HOAc
b) KOH/aq EtOH
HO
OH
CH_2OH
8α-, 8β-hydroxy-Δ9-THC
1-5%
15%
11-hydroxy-Δ9-THC
5%

Figure 10

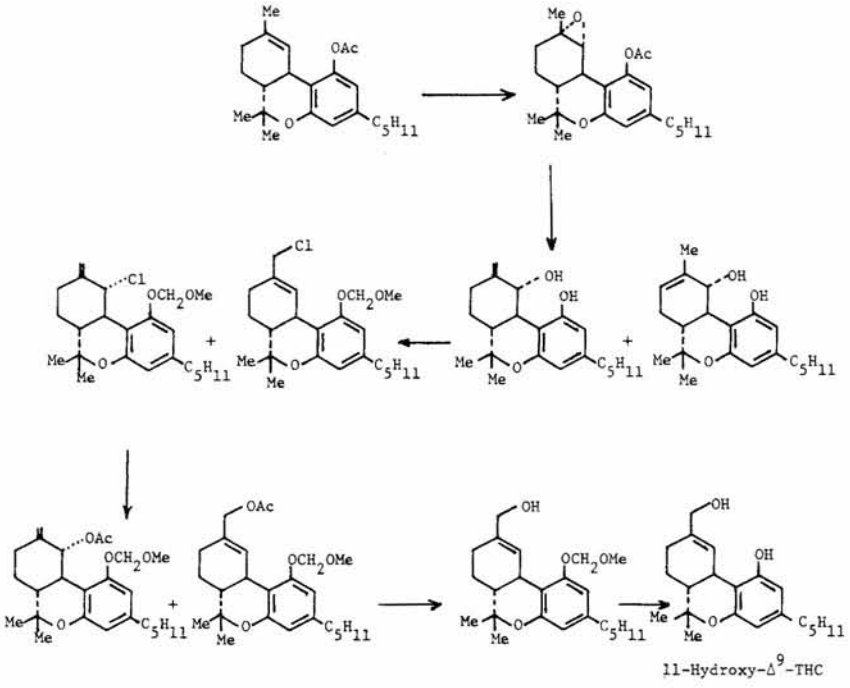


Figure 11

11-carboxy-Δ^9-THC can be prepared by careful oxidation of 11-hydroxy-Δ^9-THC. Currently there is interest in the synthesis of the 8,11-dihydroxy-Δ^9-THC because of the possible use of these compounds in the treatment of glaucoma. The synthesis[16,17] of the 8α,11-dihydroxy analog is shown in Figure 14.

METABOLISM OF Δ^9-TETRAHYDROCANNABINOL

The advances in chemical methodology discussed in the preceding sections have permitted remarkable progress to be made in studies of the physiological disposition and metabolism of the cannabinoids in recent years because of the availability of the requisite pure unlabeled and radiolabeled substrates. Because of the large volume of material to be discussed (see reviews[1-20] for more complete details) only

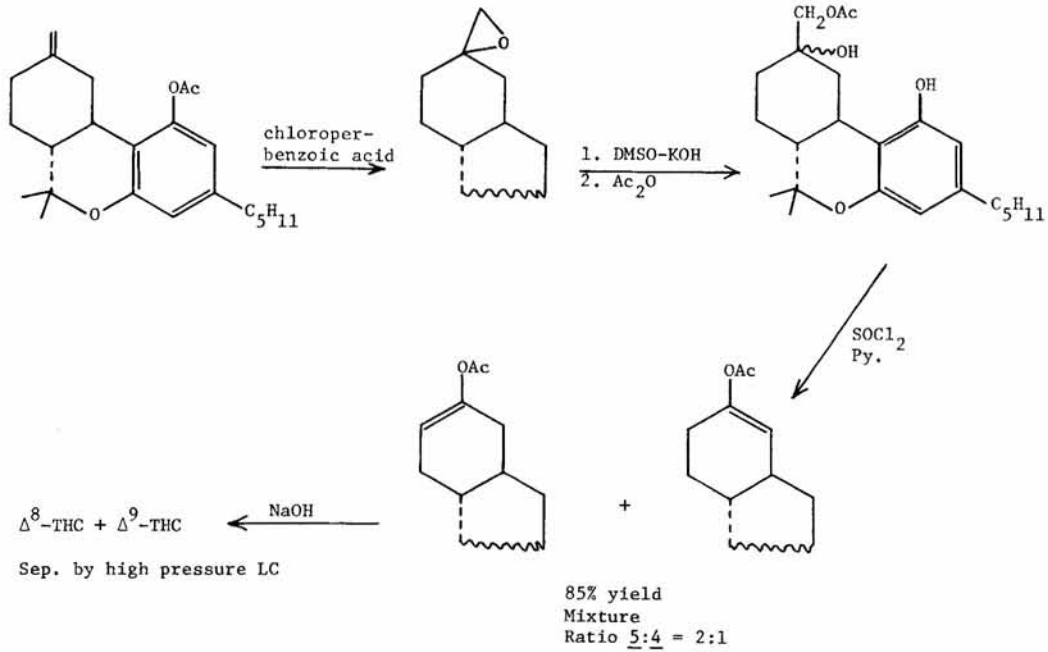

OAc
chloroper-
benzoic acid
1. DMSO-KOH
2. Ac2O
CH2OAc
OH
OH
C5H11
SOCl2
Py.
OAc
OAc
+
NaOH
Δ8-THC + Δ9-THC
Sep. by high pressure LC
85% yield
Mixture
Ratio 5:4 = 2:1

Figure 12

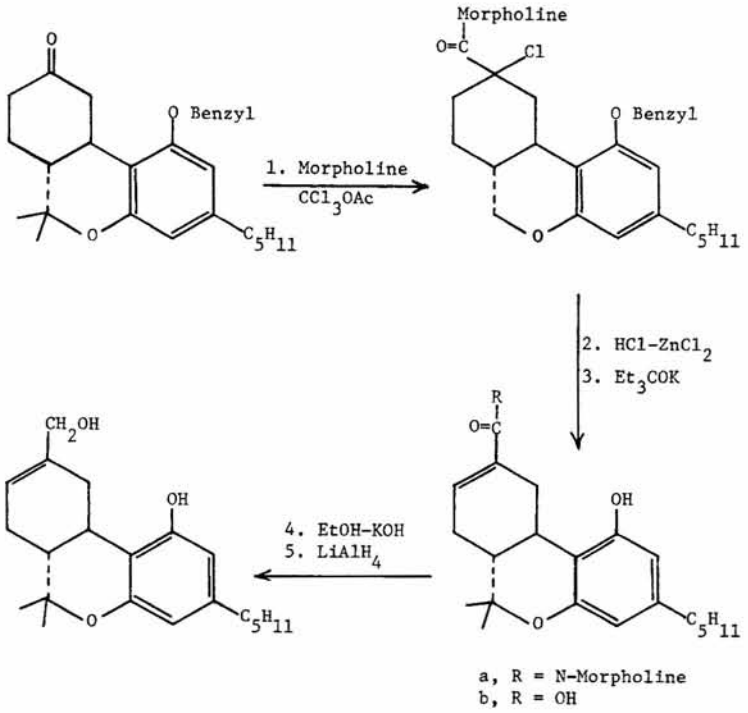


Figure 13

a few of the more important developments will be discussed in this chapter.

Figure 15 shows the sites of metabolic hydroxylation for neutral metabolites of Δ^9-THC and related cannabinoids. We have found these metabolites both *in vitro* and *in vivo*. Hydroxylation at the 11-position is the primary site of microsomal metabolism. In the Δ^9- series, in addition to 11-hydroxylation, allylic hydroxylations either at the 8α- or the 8β- have been noted[21,22]. In addition, both in the case of cannabinol[22] and more recently using monkey liver homogenates and Δ^9-THC substrate, hydroxylation at 1',2',3' and possibly 4' can occur[21]. The sidechain hydroxylation was always found in conjunction with the already noted hydroxylation at C-11. In the Δ^8-series, allylic hydroxylation occurs again at the 11-position, but in this case

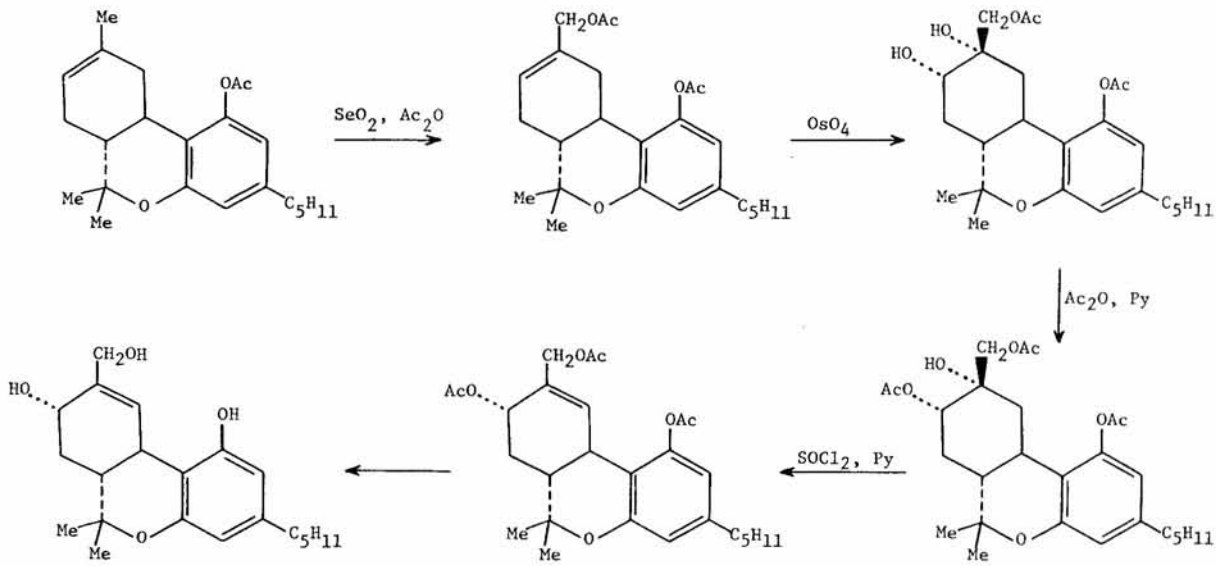

Me
OAc
Me
Me
O
C_5H_{11}
SeO_2, Ac_2O
CH_2OAc
OsO_4
HO
HO
Ac_2O, Py
AcO
$SOCl_2$, Py
CH_2OH
OH

Figure 14

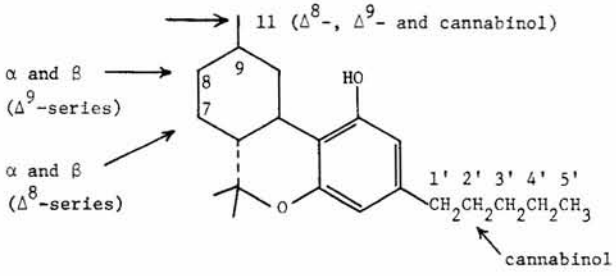


Figure 15

additional hydroxylation at the 7α- or 7β-position can occur, usually secondarily to the primary 11-hydroxylation [21,22]. Acid metabolites have usually been found after *in vivo* studies[23,24,25]. 11-Carboxy cannabinoids have been found as such in conjunction with hydroxylation at C-8 or in the sidechain as shown in Figure 16. 11-Hydroxy-Δ^9-THC is undoubtedly the intermediate for the acidic 11-carboxy metabolites. The enzyme system for this oxidation is found in the 9000 × g supernatant of liver homogenates, which also contains the microsomal hydroxylation enzymes. Recently we have demonstrated this with monkey liver homogenates, utilizing glc-mass spectrometry techniques. Under these conditions, Δ^9-THC formed a number of neutral metabolites. In addition, 11-carboxy-Δ^9-THC was also unequivocally identified[25,26], as major component *in vitro*, along with

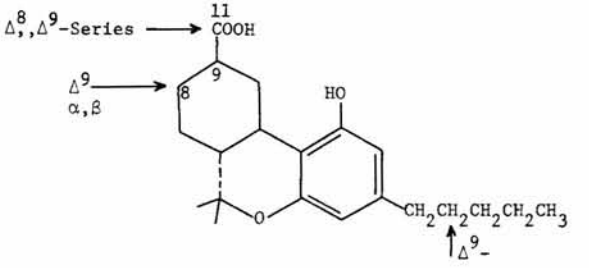


Figure 16

11-hydroxy-Δ^9-THC.

At the Research Triangle Institute we have been studying the metabolism of Δ^9-THC and related compounds in man for the last five years. During this time we have perfected techniques for the extraction and analysis of radiolabeled substrates and for the qualitative analysis of unlabeled substrates using gas/liquid chromatography-mass spectrometry (glc-ms). Charts 1, 2 and 3 summarize the procedures for the extraction and analysis of human blood, urine and feces following the administration of radiolabeled samples. Many of our studies have been done with radiolabeled Δ^9-THC administered parenterally by the intravenous route. In order to conduct these studies, a special procedure for emulsifying the water-insoluble THC had to be developed[27]. The general methodology is presented in Table 1. The neutral and acidic metabolites can be well separated by thin layer chromatography, and the zones in which these metabolites are found are scraped and then counted in scintillation counters. Figure 17 shows typical thin layer separations obtained in our laboratory. Many of these compounds have been verified by utilizing large samples of plasma or feces; after purification, glc-ms techniques have been utilized for the final identification. Table 2 shows the type of glc separation that can be obtained with these metabolites. It can be seen that Δ^9-THC is well separated from the 8-hydroxy-Δ^9-THC epimers which in turn are well separated from 11-hydroxy-Δ^9-THC. We have demonstrated that many of the THC metabolites can be identified by their glc-ms patterns[25]. When the mass spectrometric data is subjected to computerized techniques, even compounds such as the 8α-hydroxy- and 8β-Δ^9-THC, which have virtually the same retention time, can be distinguished by noting in sequence the mass spectra obtained in the various areas under the total ionization curve. In this case it became evident that 8α-hydroxy-Δ^9-THC, which has a slightly shorter retention time than the 8β-analog, is found in the early portion of the TIC plot. Since the mass spectrum of the former is significantly different from that of the 8β-analog, the two epimers can be conveniently differentiated in this manner. Using the techniques of combined glc-ms, all of the Δ^9-THC metabolites initially identified by radiolabeling techniques were unequivocally shown to be present [25,26].

With this background, we are now in a position to

Chart 1

Standard Analysis of Plasma Sample
Example of 3 ml Plasma

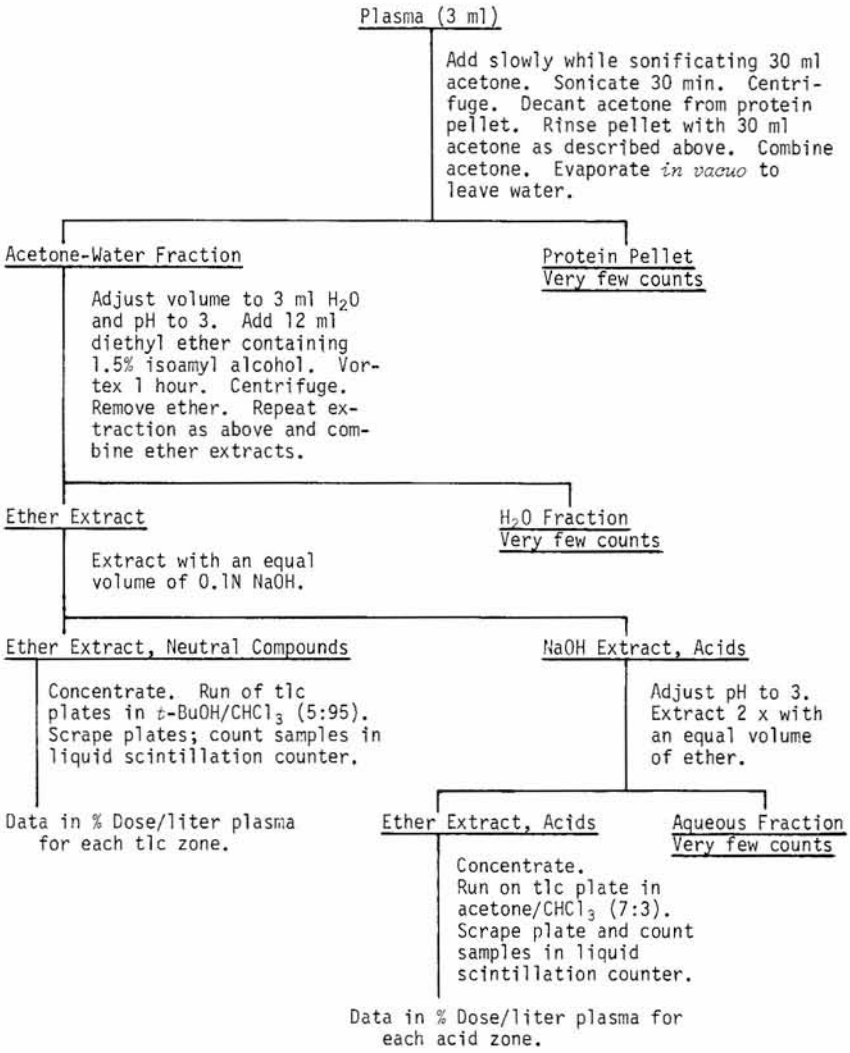


Chart 2

Standard Analysis of Urine Samples

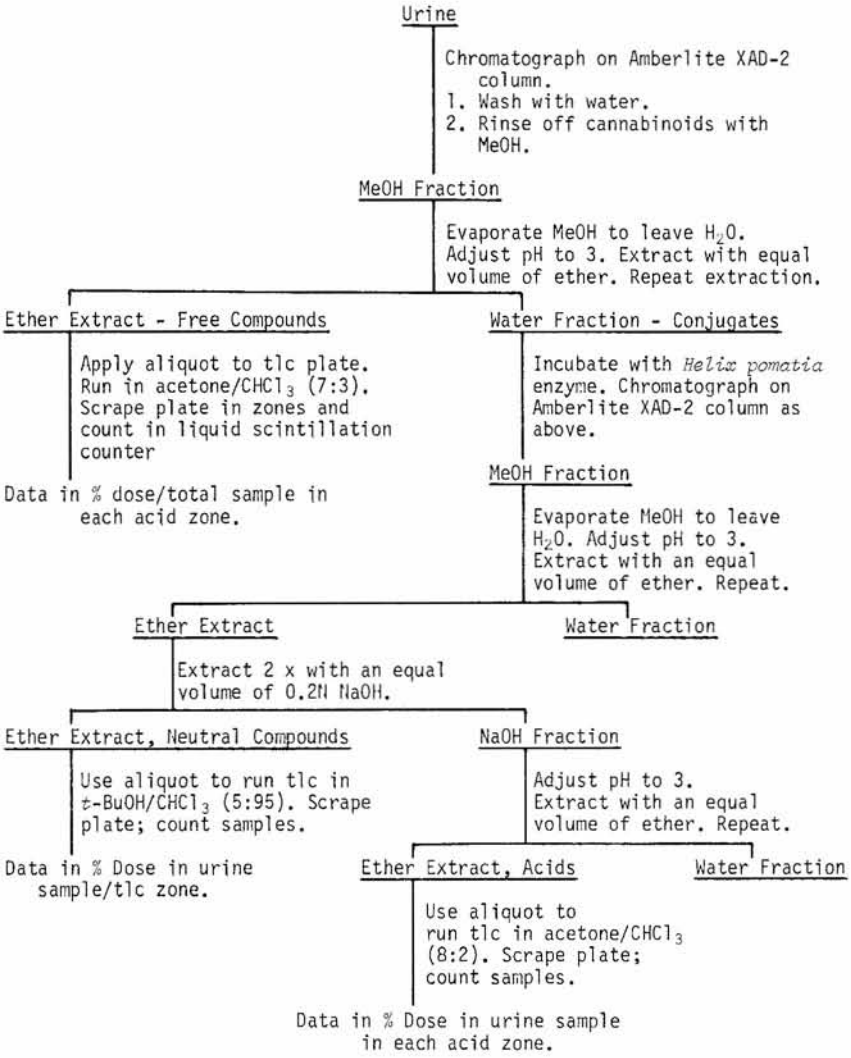


Chart 3

Standard Analysis of Feces Samples

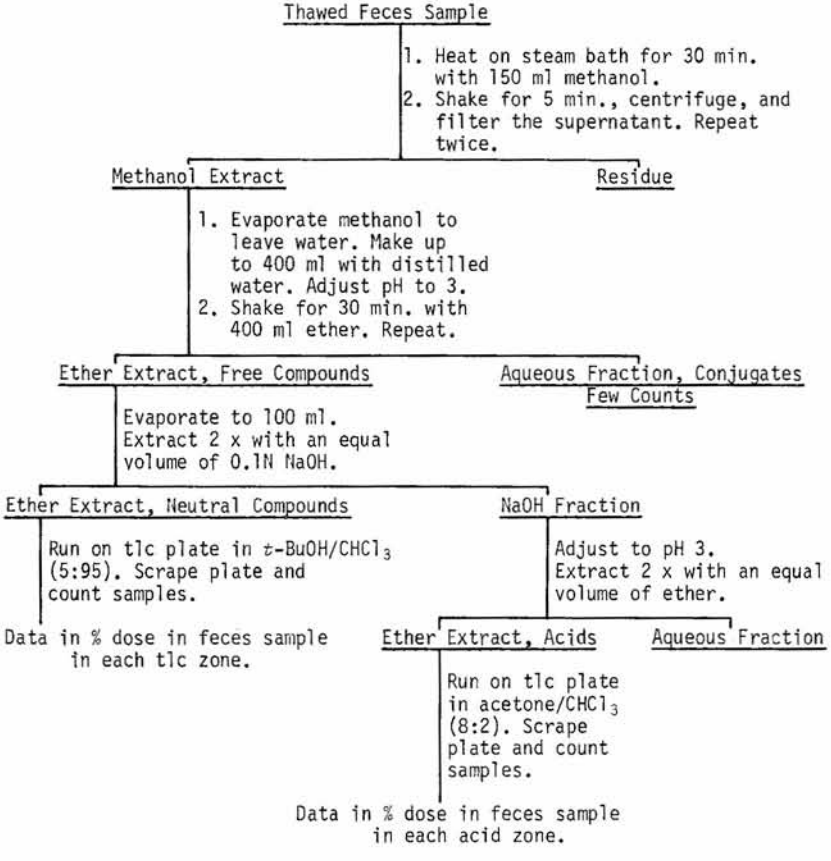


Table 1. Injectable Form of Δ^9-THC and Related Compounds

1. 10 mg THC dissolved in 0.5 ml EtOH
2. Mix with 50 ml HSA, with vigorous stirring
3. Filter through 0.22 μm Millipore filter
4. Administer dose via Harvard Constant Infusion pump at rate of 0.92 ml/min

Table 2. Glc Retention Times of TMS Ethers of Δ^9-THC and Metabolites. Column: 1% OV-17, 6 ft.

Neutrals		
TMS Ether of	220° C (min)	210 → 230° C (min)
Δ^9-THC	2.7	8.0
1'-OH-Δ^8-THC	3.3	9.3
8 -OH-Δ^9-THC	5.3	13.4
8 -OH-Δ^9-THC	5.4	13.5
3'-OH-Δ^8-THC	5.6	13.8
11-OH-Δ^9-THC	7.0	16.1
8α-11-diOH-Δ^9-THC	7.8	17.8
8β-11-diOH-Δ^9-THC	10.0	20.7
Acids		
TMS Ether of	247° C (min)	240° C (min)
11-COOH-Δ^9-THC	4.5	6.0
11-COOH, ξ-OH-Δ^9-THC	8.5	10.8
11-COOH, 8β-OH-Δ^9-THC	6.4	8.6

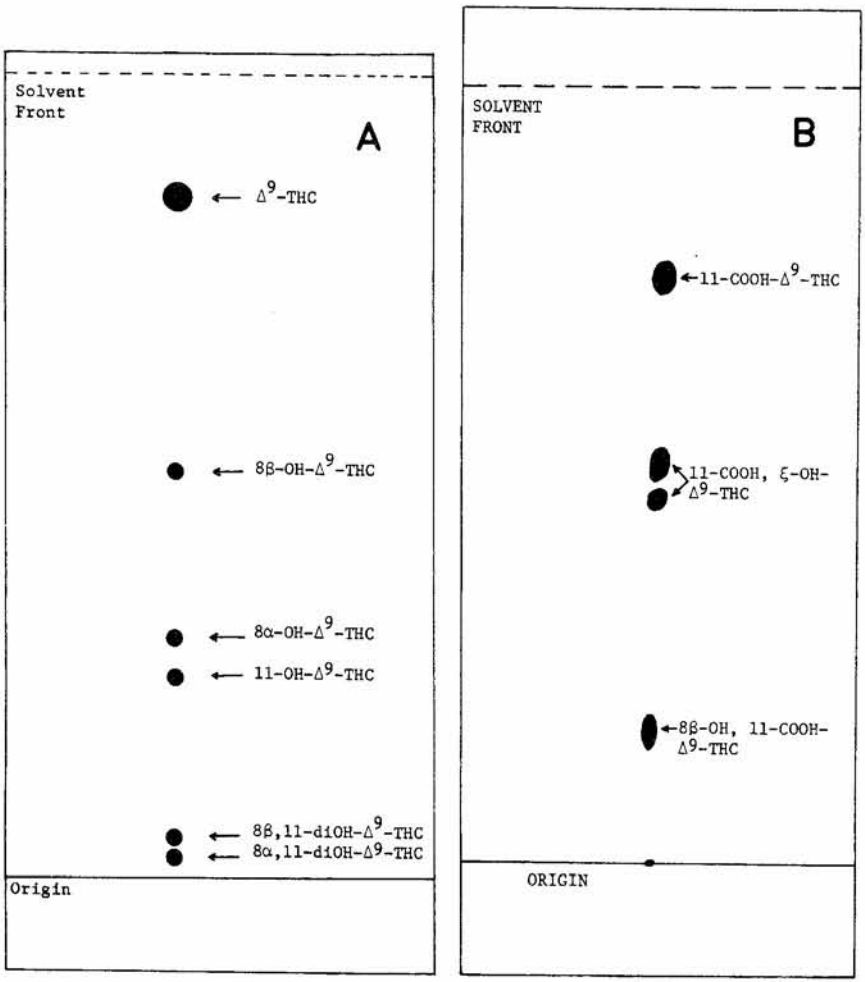


Figure 17. Tlc of cannabinoids on silica gel on glass.
A: Neutral cannabinoids; solvent: t-BuOH/$CHCL_3$ (5:95).
B: Acidic cannabinoids; solvent: acetone/ $CHCL_3$ (8:2).

discuss the metabolism of Δ^9-THC and its 11-hydroxy analog in man.

The data for total free and conjugated cannabinoids found in the plasm after IV infusion of approximately 4.6 mg of Δ^9-THC are shown in Figure 18, along with the record of the subjective psychological effects. It is apparent that the peak cannabinoid content and the psychological "high" occur at about the same time, i.e., approximately 30 minutes after administration commences. The cannabinoid content falls off rather slowly during the first 90 minutes then continues this decline over 72 hours. It will be noted that most of the cannabinoids were in the free form in the plasma, the conjugated fraction being about 5 times smaller. It can be seen that the total cannabinoid content was approximately 2% of the dose/liter plasma. This means that about 6% of the total dose may be considered to be in the blood at the time of the highest level, approximately 30 minutes after administration commenced. By 90 minutes this value had fallen rather slowly to about 0.75% dose/liter. Figure 19 shows the major constituents found in the plasma when Δ^9-THC and its 11-hydroxy metabolite are administered intravenously and also when Δ^9-THC is administered by smoking.

Turning first to the Δ^9-THC shown in Figure 19A, we see that immediately after administration of the parent substance a number of metabolites are formed. These increase, reach a maximum within 30 minutes and then slowly fall at a rate which is not greatly different from that of the parent substance. The predominant metabolite is 11-carboxy-Δ^9-THC, which has been shown by glc-ms techniques to be present in plasma, feces and urine, along with a much less well defined mixture which we call polar acids or polyhydroxy acids, which are found in equal or greater quantity. The polar acids are extractable with ether from plasma or urine and can be removed from ether with sodium hydroxide. They receive their designation "polar acids" because they do not move from the origin under conditions which readily move monohydroxylated carboxy acids. In consequence, we believe they are polyhydroxylated or possibly di- or trimeric. It is attractive to believe that they are formed by further metabolism of the 11-carboxy-Δ^9-THC, as they are formed almost simultaneously. However, this remains to be proven. Of particular interest is the low level of 11-hydroxy-Δ^9-THC. It can be seen that it is

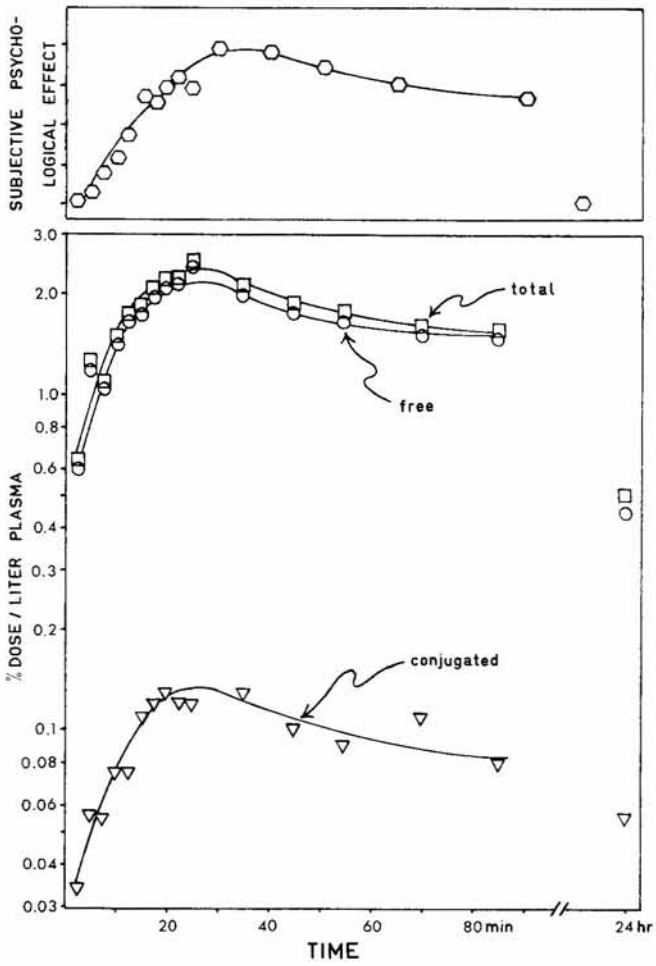


Figure 18. Subjective Psychological Effects and Total, Free, and Conjugated Cannabinoids Found in the Plasma after Administration of 4 mg Δ^9-THC via Smoking. (Average of 5 Subjects).

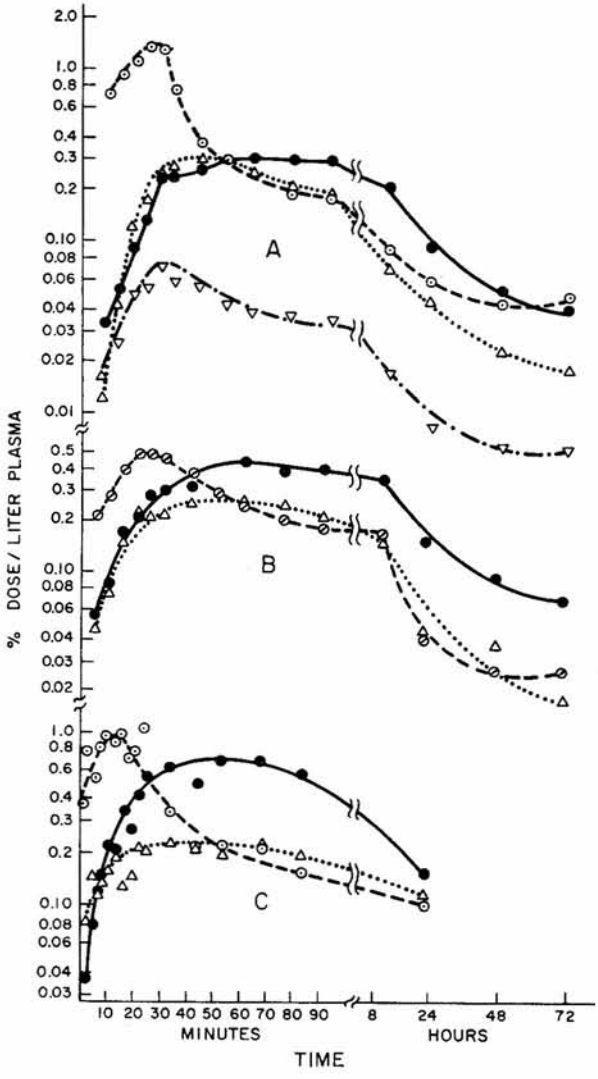


Figure 19. Metabolism of Δ^9-THC (A) and 11-OH-Δ^9-THC (B) administered intravenously and of Δ^9-THC administered by smoking (C).

A: ⊙, Δ^9-THC; △, 11-COOH-Δ^9-THC; ●, polar acids; ▽, 11-OH-Δ^9-THC.

B: ⊘, 11-OH-Δ^9-THC; △, 11-COOH-Δ^9-THC; ●, polar acids.

C: ⊙, Δ^9-THC; △, 11-COOH-Δ^9-THC; ●, polar acids.

found in amounts only one-twentieth of that of the Δ^9-THC at its peak. Not shown in Figure 19A are other minor metabolites such as 8β-hydroxy-Δ^9-THC, 8α-hydroxy-Δ^9-THC and 8,11-dihydroxy-Δ^9-THC. These compounds are found in approximately the same quantity as the 11-hydroxy metabolite.

The major metabolites in the plasma when approximately 4.8 mg of 11-hydroxy-Δ^9-THC were given by infusion to 7 volunteers are shown in Figure 19B. Again, 11-carboxy-Δ^9-THC and the polar acid fraction are the major metabolites. It is apparent that the formation of these acidic substances from 11-hydroxy-Δ^9-THC very closely resembles that found for the parent compound, Δ^9-THC. Finally, in Figure 19C are shown the data obtained when a number of volunteers received radiolabeled Δ^9-THC via the smoking route. It was estimated that the subjects actually received 4-5 mg of Δ^9-THC by this route. Again, 11-carboxy-Δ^9-THC and the polar acids were the major metabolites, with the latter now considerably exceeding the level of the simpler acid. 11-Hydroxy-Δ^9-THC is not shown in this graph but was determined and at its peak constituted only 0.03% dose/liter plasma, a level in the same general range found when Δ^9-THC was administered intravenously. It is evident that, when careful measurements are made using improved thin layer chromatography methods, 11-hydroxy-Δ^9-THC is only found in minor amounts in the plasma.

The data for total free and conjugated cannabinoids in the urine after intravenous administration of Δ^9-THC are shown in Figure 20. Over a period of 72 hours approximately 12% of the total dose administered was excreted. The major portion of the urinary constituents was conjugated and was approximately 3 times the level of the unconjugated fraction at the maximum levels found at 24 hours. The urinary constituents, both conjugated and free, were overwhelmingly acidic, with the polar acids constituting by far the major fraction, although 11-carboxy-Δ^9-THC and smaller amounts of other acids were found. Similar results were found for 11-hydroxy-Δ^9-THC and hence will not be presented.

Turning next to the data found for the analysis of the feces after Δ^9-THC and 11-hydroxy-Δ^9-THC were administered, we find that over a 72 hour period approximately 30% of the total dose was excreted in both cases. In terms of the individual cannabinoids, it was found that there was only a minor proportion of conjugated cannabinoids in the feces.

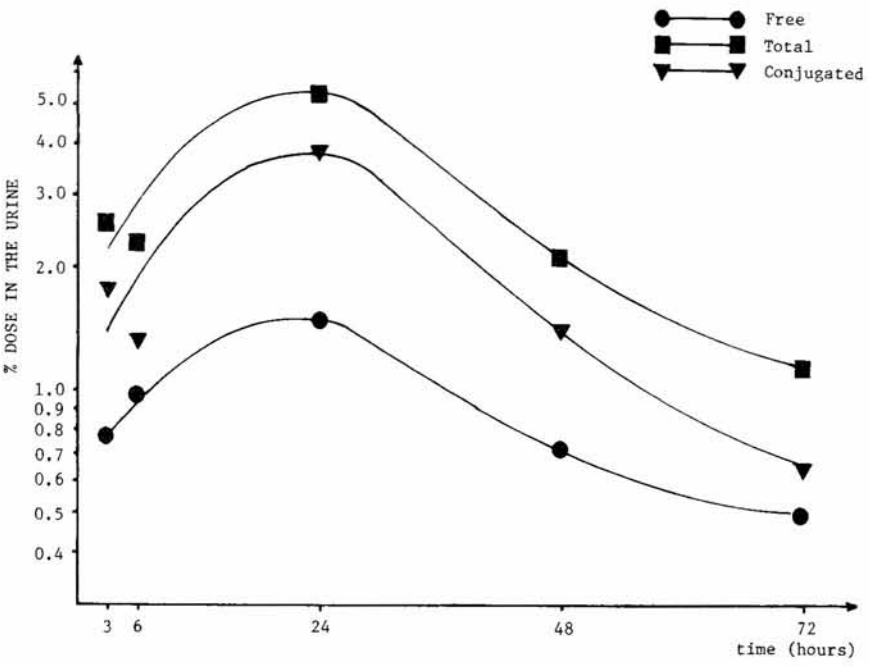


Figure 20. Total, free, and conjugated cannabinoids in urine. (Averages of 6 subjects following intravenous infusion of 4.6 mg Δ^9-THC)

The composition of the metabolites in the 24-hour feces sample is shown in Table 3. In addition to Δ^9-THC, data are also presented for 11-hydroxy- and 8β-hydroxy metabolites. The feces data show that when Δ^9-THC is administered, in marked contrast to the situation in plasma or urine, 11-hydroxy-Δ^9-THC is one of the major metabolites, along with the 11-carboxy and polar acid fraction. 11-Hydroxy-Δ^9-THC shows a rather large amount of unmetabolized starting material, i.e., about 22%, in contrast to the Δ^9- or the 8β-hydroxy compound. 11-Carboxy-Δ^9-THC and the polar acids are the major metabolites as in the case of Δ^9-THC. Again, with the 8β-hydroxy-Δ^9-THC, the polar acids are by far the major metabolites, with a compound shown to be 8,11-dihydroxy-Δ^9-THC as the next major compound found. The 11-hydroxy-Δ^9-THC found after administration of the

Table 3. Metabolites of Intravenously Infused Δ^9-THC, 11-OH-Δ^9-THC and 8β-OH-Δ^9-THC.

Free Cannabinoids in 24 hr feces	Substrate		
	Δ^9-THC	11-OH-Δ^9-THC	8β-OH-Δ^9-THC
	(% of total cannabinoids)		
Polar acids	29	41	72
Intermediate Acids	9	10	-
8,11-diOH-Δ^9-THC	Minor	Minor	24
11-COOH-Δ^9-THC	29	28	-
11-OH-Δ^9-THC	21	22	-
8β-OH-Δ^9-THC	5	-	4
Δ^9-THC	8	-	-

Δ^9- parent substance is most likely formed by metabolism in the liver and not by microorganisms in the gut. This is indicated by the fact that administration of Δ^9-THC rectally to several volunteers and then analysis after 24 hours showed that virtually no change had occurred in the starting Δ^9-THC. It is conceivable, of course, that the 11-hydroxy-Δ^9-THC is conjugated in the liver and is excreted in the bile in this form and is then hydrolyzed during passage through the small and large intestines. Our point is that 11-hydroxylation occurs via the liver microsomal enzyme system.

We interpret the data previously presented to show that the main course of metabolism of Δ^9-THC proceeds via its 11-hydroxy analog as the key compound in the metabolic series. This allylic hydroxylation proceeds rapidly and readily. Subsequently, additional hydroxylation at 8α- or 8β- positions or in the sidechain may occur. All of these metabolic hydroxylations are brought about by the liver microsomal enzyme system. In addition, another enzyme system can convert the 11-hydroxy-Δ^9-THC to the corresponding 11-carboxy-Δ^9-THC, presumably via the common alcohol

dehydrogenase mechanism. Although 11-hydroxy-Δ^9-THC is readily formed both *in vitro* and *in vivo*, it is evident from our data that little is found in the plasma. Apparently, this metabolite is preferentially excreted via the bile and, as noted earlier, constitutes a major metabolite found in the feces. Because 11-hydroxy-Δ^9-THC is found in only relatively small quantities in the plasma, we deduce that large amounts of this compound cannot get into the brain. From our studies[28] in which it was found that Δ^9-THC and 11-hydroxy-Δ^9-THC have very similar biological activities in man, we believe that 11-hydroxy-Δ^9-THC cannot be the only active form. We deduce this since, as stated earlier, it is found in such very low quantities in the plasma, and if this indeed were the only active form then one would assume it would have to be biologically very potent. Accordingly, it is our belief that a number of cannabinoids, including Δ^9-THC, 11-hydroxy-Δ^9-THC and several others, including even cannabinol, can be active in the proportions in which they are received at an appropriate receptor site, which is presumably in the brain.

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