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Marihuana Chemistry

Recent advances in cannabinoid chemistry open the area to more sophisticated biological research.

Raphael Mechoulam

The various *Cannabis sativa* preparations are the most widely used illicit drugs in the world. Marihuana, hashish, dagga, charas, bhang, and others, are estimated to be consumed, mostly for hedonistic purposes, by 200 million to 300 million people. In recent years the smoking of marihuana and hashish has become prevalent in some sectors of the young middle-class society of North America and, to a lesser extent, that of Western Europe. This circumstance has brought forward vociferous advocates for the legalization of the drug and equally vehement opponents of any basic change of the legal position. The differences of opinion center on the short- and long-term effects, both physiological and mental, of the use of *Cannabis*. It is a sad truth, however, that, in spite of the voluminous literature on the subject (*1*), critical scientific evaluations of the different aspects of the problem are few. This lack of pharmacological and clinical data is due, to a large extent, to the fact that until very recently the state of our chemical knowledge of these substances was poor. The use of crude marihuana, or extracts from it, for detailed and reproducible biological work has many disadvantages, the major one being the notorious variability of *Cannabis* preparations. The amount and ratio of the components in the drug are a function of botanical and cultivation factors, as well as of the mode of preparation of the crude drug, and of the conditions (such as light, heat, and duration) of storage (*2, 3*). Hence, a firm chemical basis is a prerequisite for quantitative biological work for which pure and well-defined substances are needed. However, until 1964 (*4*) the major active component had not been isolated in a pure form and its structure had been known only in a general way. Numerous cannabinoids (*5*) were known to be present in the resin and in other parts of the plant, but only a few had been isolated before 1963 and the structure of only one, the psychotomimetically inactive cannabinol, had been fully elucidated (*2*).

This lack of knowledge contrasts sharply with the situation concerning morphine and cocaine, the two other major drugs which originate from natural sources and which are used for illicit, nonmedical purposes. These two alkaloids were already well known in the last century, and hence their biological and clinical properties were extensively investigated.

In the last few years intensive chemical investigations have considerably clarified the rather complex chemistry of marihuana. Most natural cannabinoids have been isolated and purified; their structures have been elucidated; syntheses of most of the components have been accomplished; analytical methods have been developed, and metabolic work has been initiated. However, there has been no parallel advance in the pharmacologic, psychologic, or clinical aspects of cannabinoids.

In this short review the state of cannabinoid chemistry is summarized for the research biologists, and perhaps some will be enticed to fill the gaps of knowledge concerning the nature of the physiological and psychological activity of these compounds. Details of the structure elucidations and of syntheses will not be presented. Considerations of reactions and their mechanisms that are mainly of chemical interest, though important and fascinating, are not discussed here because they have already been reviewed (*2, 6*).

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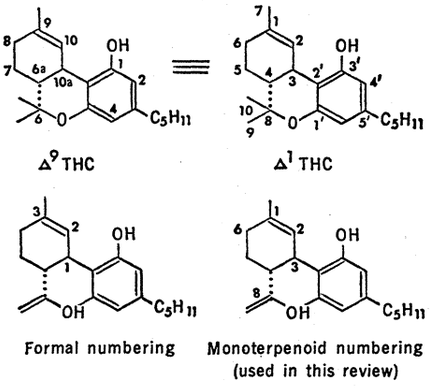
Nomenclature

Two numbering systems for this class of compounds are in use today. In one the formal chemical rules for numbering of pyran-type compounds are used for the tetrahydrocannabinols. For cannabinoids which are not pyrans, this numbering is not applicable; hence on passing from one compound to another in this series a carbon atom frequently has its number changed. The second nomenclature has a biogenetic basis; the cannabinoids are regarded as substituted monoterpenoids, and the numbering is the accepted one for compounds of this class. It can be used for all cannabinoids; hence, it has the advantage that a carbon atom in the molecule retains the same number in most chemical transformations.

As an example, in the following formulas, the major natural tetrahydrocannabinol (THC) and a major inactive component, cannabidiol, are numbered according to both systems. The monoterpenoid numbering is only used throughout this review.



Isolation Procedures

The full separation of the closely related natural cannabinoids is a tedious undertaking. Most, though not all of them, are soluble in petroleum ether. By repeated extraction with this solvent all the active material in the crude resin can be dissolved. The remaining inactive material contains mostly phenolic polymers, small amounts of cannabielsoic acids which can be extracted by benzene, and other unidentified compounds. The petroleum ether extract has been separated into acidic and neutral fractions, both of which, on column chromatography (*2*, *8*) yield numerous cannabinoids and some unidentified sesqui- and triterpenes. The important Δ^1-tetrahydrocannabinolic acid A (Δ^1-THC acid A) (*7*) is only partially taken up by base from a petroleum ether extract. In part it forms a tar, together with some triterpenoid acids, and remains on the interface of the organic and aqueous media (*8*). Though inactive in itself, Δ^1THC acid A (*7*) is converted, when it is smoked, into the active Δ^1THC (*4*). Therefore the erratic behavior of Δ^1THC acid A on basic extraction should be taken into account, particularly in analytical work aimed at determining the amount of potentially active material on smoking.

The petroleum ether extract has also been separated by repeated countercurrent distributions (*9*). A certain disadvantage in this technique is that the high-boiling dimethylformamide used in the solvent system is difficult to remove and may contaminate some of the oily constituents.

Natural Cannabinoids:

The Tetrahydrocannabinols

Until recently (*4*), it was generally assumed that the active principles of *Cannabis* were an unidentified mixture of isomers of tetrahydrocannabinols. Work during the last few years has shown that there is, in fact, one major natural THC, the Δ^1 isomer, but that varying, usually small, amounts of the $\Delta^{1(6)}$ isomer may be present.

A tetrahydrocannabinol was isolated in an impure form in 1942 (*10*) when a gross structure was suggested for it. In 1964, a crystalline derivative was prepared from which pure Δ^1THC could be obtained, apparently for the first time; its structure and stereochemistry were elucidated, and a partial synthesis was achieved (*4*). Pure Δ^1THC showed strong activity in the ataxia test in dogs (*4*, *11*) and had psychotomimetic activity in humans (*2*). The structure of Δ^1THC, as well as that of the other cannabinoids isolated in recent years, was determined by extensive use of modern physical techniques such as nuclear-magnetic-resonance spectroscopy (*4*, *12*) and mass spectrometry (*13*).

The oily Δ^1THC is a labile substance. It is easily isomerized by acids to $\Delta^{1(6)}$THC (*14*, *15*). In the presence of air it is slowly oxidized to cannabinol and, perhaps, compounds of higher molecular weight. The latter can be formed through phenolic oxidation. In the presence of base, this oxidative phenolic coupling is accelerated (*8*). Hence, a Δ^1THC sample which on gas-liquid chromatography analysis may seem pure can, in actuality, contain considerable amounts of nonvolatile polymers.

The major psychotomimetic principle in all *Cannabis* preparations is Δ^1THC, and it can apparently reproduce fully the effects of the crude drug in animals and humans. The oral effective dose in humans is about 50 to 200 micrograms per kilogram of body weight and the dose when smoked is 25 to 50 μg/kg (*2*, *16*, *17*). The burning process destroys part of the THC, therefore the actual dose absorbed on smoking may be considerably lower.

The $\Delta^{1(6)}$THC isomer is a minor active compound in marihuana (*15*). Though in some fresh marihuana samples $\Delta^{1(6)}$THC may represent up to 10 percent of the combined THC content (*15*), usually the percentage is considerably lower. Analyses of Lebanese hashish over a period of 5 years have shown that $\Delta^{1(6)}$THC did not exceed 0.5 to 1 percent of the amount of Δ^1THC (*8*). In 14 samples of Mexican marihuana (*18*) the range of the ratio of Δ^1THC to $\Delta^{1(6)}$THC varied from 99.9 : 0.1 to 98.6 : 1.2.

It has been suggested (*19*, *20*) that on storage, as well as on smoking, Δ^1THC may be converted into $\Delta^{1(6)}$THC. Later work has shown that this isomeri-

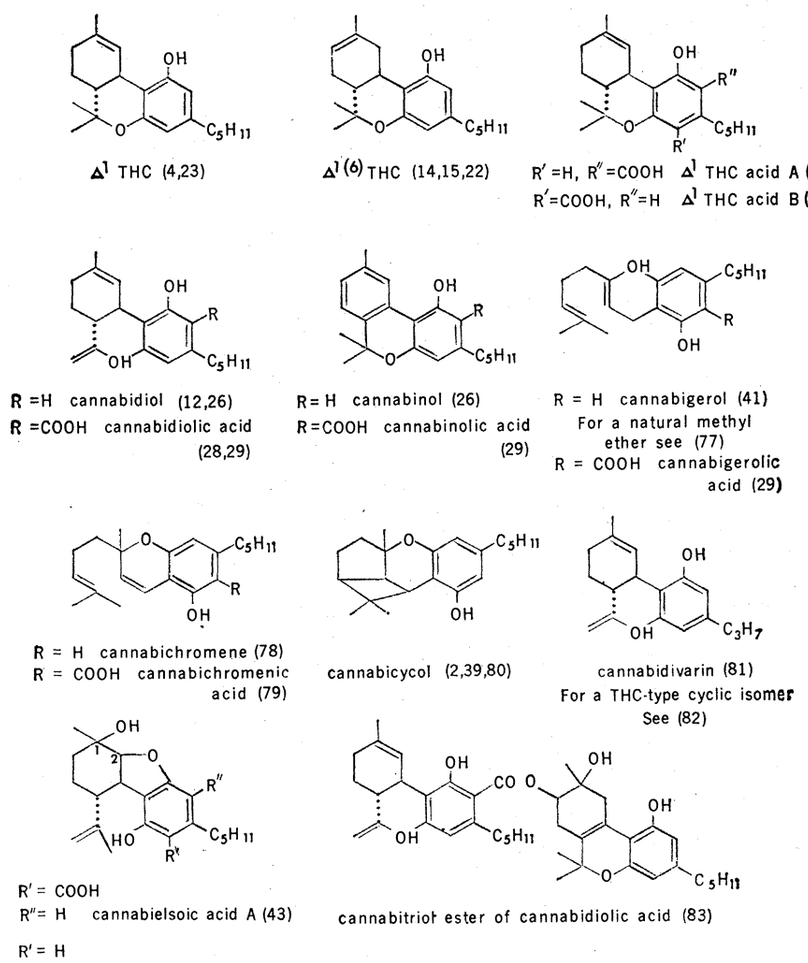


Fig. 1. The natural cannabinoids.

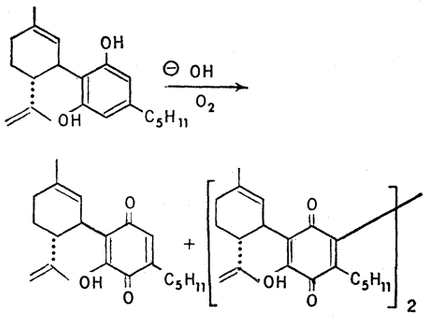
zation is of minor importance under these conditions (*18*, *21*). In aged *Cannabis*, Δ^1THC indeed decreases, but in absolute figures $\Delta^{1(6)}$THC does not increase. As the rate of deterioration (oxidation) of Δ^1THC is higher than that of $\Delta^{1(6)}$THC (*8*), the observed change does not necessarily indicate a conversion of Δ^1THC into $\Delta^{1(6)}$THC.

The $\Delta^{1(6)}$THC isomer was first prepared semisynthetically in the early 1940's (*22*). Its structure was partially determined at the time, and it was fully established by modern techniques in 1966 (*13–15*). The absolute configuration of Δ^1THC and $\Delta^{1(6)}$THC at the chiral centers carbon-3 and carbon-4 is R (*23*). Minor differences have been found in the pharmacological activity of Δ^1THC and $\Delta^{1(6)}$THC in the few systems tested so far (*11*, *24*, *25*). In the early 1940's (*26*) $\Delta^{1(6)}$THC was administered to human volunteers and thus may represent the first demonstration of hashish-like activity of a THC of known chemical structure in man.

Other neutral natural cannabinoids. The structures of, names of, and literature references for the neutral natural cannabinoids identified so far are given in Fig. 1. Except for the THC's, the components are psychotomimetically inactive and do not seem to exert potentiating or other effects (*24*). This aspect has not as yet been adequately investigated. It is known, however, that cannabidiol potentiates barbiturates (*27*). Cannabidiol and cannabigerol are antibiotic in vitro (*28*, *29*). The latter is inactivated by serum (*29*).

Many natural cannabinoids are unstable to acids. The reactions in Fig. 2 are typical.

Cannabidiol and cannabigerol in base in the presence of oxygen are oxidized to the monomeric and dimeric hydroxyquinones indicated below (*30*). The



anions of the oxidized compounds have a deep violet color. This is the basis of the Beam color reaction used for identification of *Cannabis*.

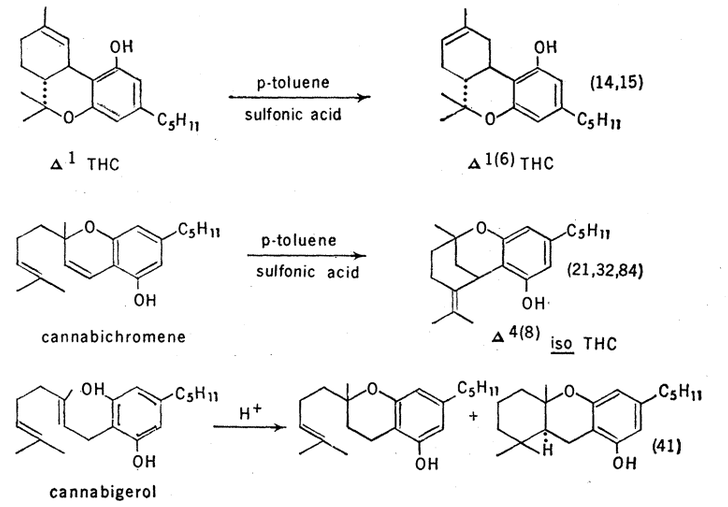


Fig. 2. Typical reactions of cannabinoids with acids.

Natural cannabinoid acids. The structures of, names of, and references for the known cannabinoid acids are given in Fig. 1. The cannabinoid acids are psychotomimetically inactive. Cannabidiolic and cannabigerolic acids are antibiotic to Gram-positive bacteria in vitro (*28*, *29*).

The cannabinoid acids are rapidly converted when heated, and slowly when stored, into the respective neutral components (*28*, *31*). These processes are of major importance. As has been mentioned above, when the inactive THC acids (both A and B) are smoked, they give active Δ^1THC. This is one of the reasons for the higher activity of marihuana following smoking as compared with ingestion.

Cannabidiolic acid is the predominant cannabinoid in nature. In hemp grown for fiber it is apparently the only major constituent of this type (*28*). *Cannabis* plants grown for the psychotomimetic resin have less of this constituent and more THC (*3*).

It is still debated (*6*) whether the neutral cannabinoids are authentic natural products or whether they are artifacts formed by decarboxylation of the respective cannabinoid acids. It seems that *Cannabis* grown in northern countries possesses little if any neutral cannabinoids, whereas that grown in tropical or subtropical countries undergoes at least some decarboxylation in the plant. The crudity of illicit preparation of the drug itself (marihuana, hashish) apparently causes further decarboxylation of the acids.

The amounts of cannabinoids vary considerably from sample to sample. A recent analysis (*8*) of a 10- to 15-month-old hashish sample of Lebanese origin showed the following: cannabidiol (1.4 percent), Δ^1THC (1.4 percent), cannabinol (0.3 percent), cannabigerol (0.3 percent), cannabicyclol (0.1 percent), cannabichromene (0.1 percent), cannabidiolic acid (4.5 percent), Δ^1THC acid A (3.1 percent), cannabinolic acid (0.25 percent), and cannabielsoic acids (0.08 percent). A partial analysis of hashish from a different batch (age unknown) showed the presence of cannabidiol (2.5 percent), Δ^1THC (3.4 percent), cannabidiolic acid (3.2 percent), and Δ^1THC acid A (1.4 percent). The general experience in my laboratory over the last 5 years indicates that the concentration of cannabidiol and cannabidiolic acid in Lebanese hashish is 5 to 7 percent, while that of Δ^1THC and Δ^1THC acid A is 4 to 5 percent.

Biogenesis

A general biogenetic scheme has been suggested (*2*). It is slightly modified, as shown in Fig. 3.

The hydroxycannabigerol intermediate (Fig. 3, part shown in brackets) is not necessarily found as such in the plant. It only indicates that cannabigerol has to be brought to a higher state of oxidation.

Cannabichromene (*32*), cannabichromenic acid (*33*), and cannabicyclol (*34*) are optically inactive. This may indicate that either the cyclization leading to these compounds passes through a symmetric intermediate such as "intermediate A," or that the formation of these compounds involves a nonenzymic process.

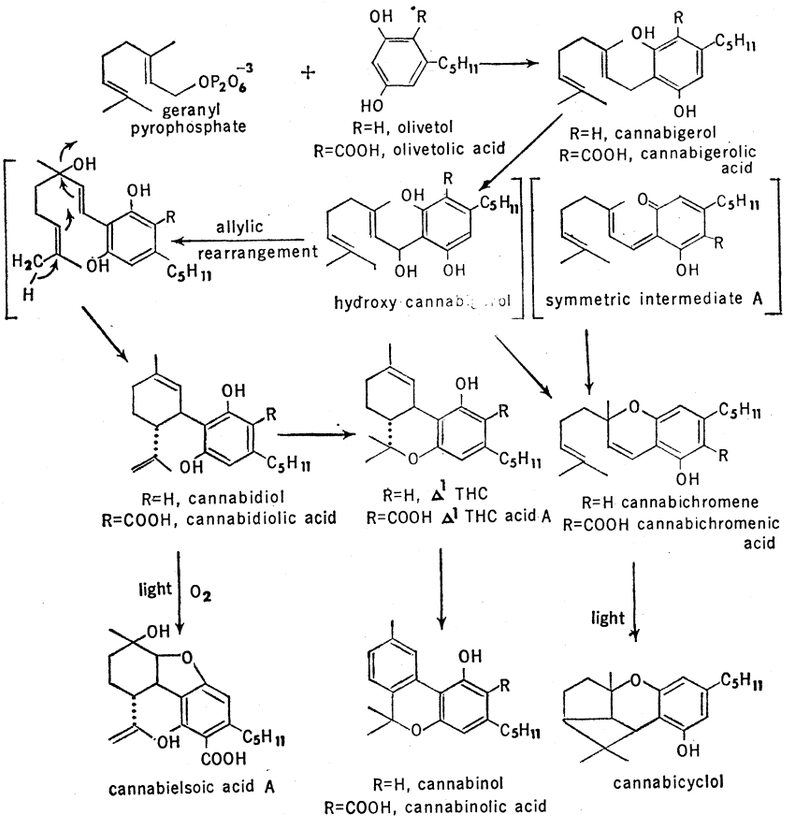


Fig. 3. Proposed biogenesis of cannabinoids.

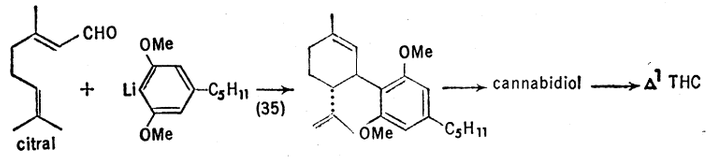


Fig. 4. First total synthesis of *dl*-Δ^1THC.

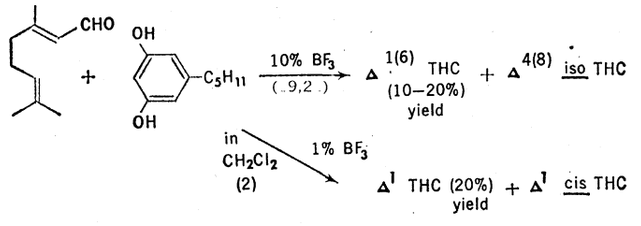


Fig. 5. Practical syntheses of *dl*-Δ^1THC and *dl*-$\Delta^{1(6)}$THC.

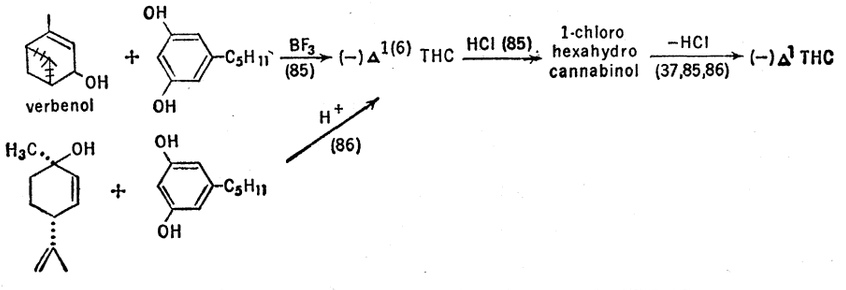


Fig. 6. Syntheses of optically active Δ^1THC and $\Delta^{1(6)}$THC.

The presence of acidic and neutral cannabinoids suggests a few biogenetic possibilities: (i) the neutral components are formed by decarboxylation of the respective carboxylic acids only or (ii) parallel biogenetic sequences exist in the plant for both sequences.

Although experiments on biogenesis with labeled precursors have been performed in several laboratories, these have been disappointing in view of the low incorporation of radioactive material in the isolated cannabinoids.

Syntheses of Cannabinoids

The first cannabinoid to be synthesized was the inactive cannabinol (*26*). An unexpected result of this work was that the intermediate Δ^3THC proved to have hashish-like activity. In that the natural active constituents did not become available for another 25 years Δ^3THC has been used in most biological work as the standard compound.

The first total synthesis of *dl*-Δ^1THC and *dl*-cannabidiol (and formally of $\Delta^{1(6)}$THC too) was reported in 1965 (*35*). It is described in Fig. 4. This synthesis is of little practical value: The yield is low, it is not stereospecific, and it gives a racemic mixture. Numerous groups of investigators have since improved and modified this route or accomplished entirely new syntheses (*2, 36, 37*). It seems that the most facile preparations of *dl*-Δ^1THC and $\Delta^{1(6)}$-THC are those described in Fig. 5. For biological work, however, syntheses leading to pure, natural *l* modifications are, of course, to be preferred. The syntheses described in Fig. 6 seem to be the methods of choice with respect to yields, simplicity of procedure, and purity of *l* products. For special purposes, such as labeling or structure modifications, some of the other syntheses are of importance (see *38*).

Until now, synthetic THC has not been used for illegal purposes. This is due to the ready availability of marihuana. If the strict measures taken by the U.S. government to curb marihuana imports succeed, THC synthesis may become a worthwhile proposition for organized criminal groups. The best way to prevent this may be to impose national and international controls on olivetol and related alkyl resorcinols.

Cannabichromene (*39, 40*), cannabicyclol (*34, 39*), cannabigerol (*41*), as well as THC acid A, cannabidiolic acids, cannabigerolic acid (*42*), and

cannabielsoic acid A (*43*) have been synthesized. The practical importance of these syntheses is that they make these compounds available in pure form for eventual study of their synergistic or other effects with the THC's.

Structure Activity Relations

The accidental discovery that the unnatural Δ^3THC possesses hashish-type activity (*26, 44*) led in the early 1940's to the synthesis of numerous derivatives that were tested either by the dog-ataxia test or the Gayer corneal-areflexia test. The results of these two tests are only roughly comparable (*27*).

In the Δ^3THC series (Fig. 7) it was found that changes in the side chain of the aromatic moiety can bring considerable increase in activity in both the dog-ataxia and the Gayer tests. The most active compound proved to be the one with a dimethylheptyl side chain [R=$CH(CH_3)CH(CH_3)C_5H_{11}$]. This compound (or rather mixture of isomers) was reported to be as much as 500 times more active than the standard Δ^3THC (*27*). A sample of the same compound from a different synthetic batch, however, was only about 60 times more active than Δ^3THC (*27*). In view of the three chiral centers in the molecule, it is possible that these variations are due to different proportions of isomers in the batches tested. More recently, the eight possible isomers have been obtained in optically pure form (*45*). The report on their biological activity is awaited with interest.

With the development of syntheses of the natural THC's, modifications were made on these molecules. As with Δ^3THC, changes in the side chain caused sharp increase in activity in rhesus monkeys (*8, 11, 24*). The most active compound so far is Δ^1THC, in which the side chain is dimethylheptyl. The active dose is at least five times lower than that of Δ^1THC, and the activity is prolonged from a few hours to a few days.

Substitution of an aromatic hydrogen in $\Delta^{1(6)}$THC by a methyl group retains the activity; a carbomethoxyl group or a hydroxyl group in the same position eliminates it (*8, 24*).

Acetylation of the free hydroxyl in either $\Delta^{1(6)}$ or Δ^1THC apparently reduces the activity (*24*). This observation, which has yet to be confirmed, indicates that the activity is somehow related to the free phenolic group. This is a rather sad state of affairs, for it suggests that derivatives formed through the phenolic group probably will not have the same activity as the natural THC's. One of the major difficulties of cannabinoid pharmacology and biochemistry is the insolubility in water of the THC's, and the easiest way to circumvent this difficulty would have been to prepare water-soluble derivatives via the phenolic group. However, in view of the above results this problem will probably have to be solved not by structure modification of the molecule, but rather by a suitable solvent formulation.

Few modifications on the terpene ring have been reported and tested. A mixture of the two hexahydrocannabinols is active (dog ataxia) (*27*); 2α-hydroxy-$\Delta^{1(6)}$THC is almost as active as $\Delta^{1(6)}$THC, but the isomeric 2β-hydroxy-$\Delta^{1(6)}$THC is not active (in monkey tests) at 0.5 mg/kg (*8, 24*).

None of the synthetic *iso*-THC's (*32*) tested so far in monkeys have shown any activity (*24*). The $\Delta^{1(7)}$THC is likewise inactive (*37, 46*) when tested in monkeys (*47*). The aza-THC (Fig. 7; **1**,R=C_5H_{11}) parallels the natural THC's in activity on the central nervous system (*49*). Homologs of this aza-THC (see formula **1**) in which the side chain varied [R= $-CH(CH_3)(CH_2)_4CH_3$; or R= $-CH(CH_3)(CH_2)_{11}CH_3$; or other] were also active. Two other aza-THC's are known, formulas **2** and **3** (*48, 50*). A group of related compounds (**4**) with central nervous system activity have been described (*51*). Several are shown in Fig. 7. An inactive steroidal THC has been prepared (*52*). A position isomer of Δ^3THC with a dimethylheptyl side chain (**5**) was found to be physiologically inactive (*53*), though earlier related compounds had been shown to be analgetic (*54*).

Few of the compounds have been tested in humans. It was found in the early 1940's that on oral administration 15 mg of $\Delta^{1(6)}$THC (prepared by a semisynthetic route from natural cannabidiol) is equal in psychotomimetic activity to 60 mg of synhexyl- or to 120 mg of Δ^3THC (*26, 55*). These results conform in a general way to the animal data. Recent results in humans are less clear-cut. As was mentioned above, Δ^1THC is active on smoking and on ingestion (*16, 17*). Synhexyl- shows strong activity on ingestion (*17*) but Δ^3THC is inactive up to 400 μg/kg (smoking) (*16*). The latter compound probably has to be tested again and at higher doses. It will be most embarrassing if Δ^3THC, which served as a standard in animal testing of cannabinoids for many years turns out to be inactive in humans.

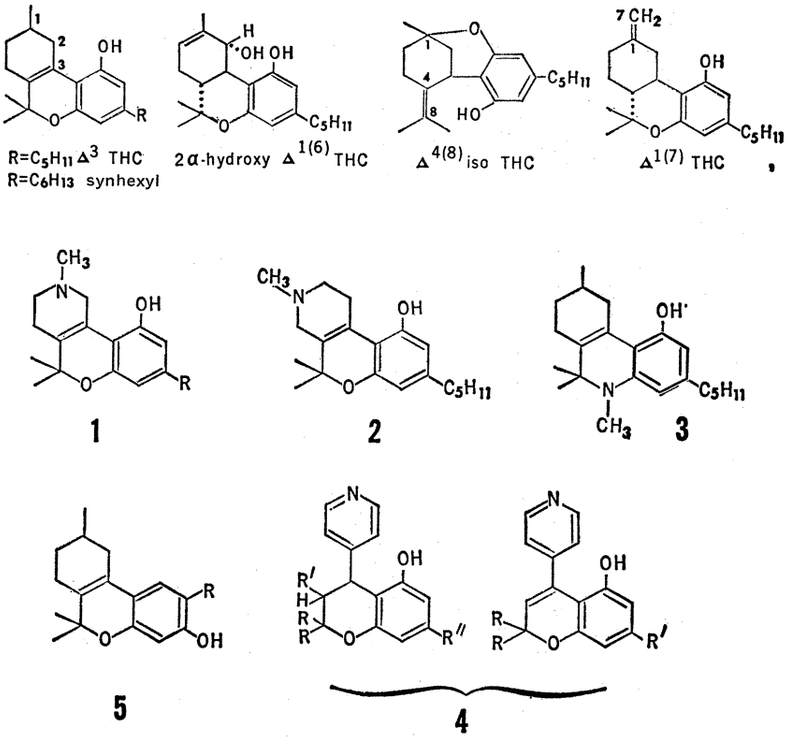


Fig. 7. Structures of several analogs.

Analytical Aspects

Color tests have been used for many years for the identification of *Cannabis*. Their specificity has been investigated by the United Nations Laboratory in Switzerland (*3*). The Beam test—a purple color with 5 percent ethanolic potassium hydroxide—is relatively specific. Out of 120 plant species examined, only two, *Rosmarinus officinalis* and *Salvia officinalis*, give a weakly positive reaction; out of 48 pure substances of vegetable origin (monoterpenes, sesquiterpenes, and aromatic compounds), only one, the quinone juglone, develops a color, close to that of the Beam test. The chemical basis of this test is oxidation of cannabidiol, cannabigerol, and their acids to hydroxyquinones, the anions of which are violet (*30*). Δ^1-Tetrahydrocannabinol gives a negative Beam test. The Duquénois-Negm reaction—a purple color with vanillin, acetaldehyde, and ethanol in hydrochloric acid—though less specific than the Beam test is more sensitive. Application of these two tests in parallel offers a high probability for the positive identification of the drug.

Thin-layer chromatography methods are used for qualitative analysis of *Cannabis*; silica gel impregnated with dimethylformamide and with cyclohexane as eluent are frequently used (*56*). When sprayed with blue tetrazolium (di-*o*-anisidinetetrazolium chloride) cannabidiol gives an orange spot, Δ^1THC a red one, and cannabinol a violet one (*57*). It has been reported, however, that the R_F values in this thin-layer chromatography system are greatly affected by the grade of dryness of the impregnating solvent (*58*). In a simpler system chromatoplates of silica gel and elution with a mixture of pentane and ether (88 : 12) are used. A potassium permanganate solution was used as a developing agent (*29*). In Table 1 the R_F values of some neutral cannabinoids are presented. Numerous other systems have been suggested (*58, 59*).

Gas-liquid chromatography is the method of choice for *Cannabis* analyses. It was first applied by a group at the Canadian Food and Drug Directorate in the early 1960's (*60*) and numerous systems have been tested since then. The columns in use today are SE 30 (*60, 61*), XE 60 (*62*), Carbowax 20M (*63*), and OV 17 (*18, 64*). Good separation between cannabinol, Δ^1THC, cannabidiol, cannabichromene, and cannabigerol is observed with any one of these columns. However, $\Delta^{1(6)}$THC and Δ^1THC have close retention times, and, hence quantitative differentiation between these two THC's is frequently difficult. In Table 1 the retention times of some neutral cannabinoids on an OV-17 column are presented. All cannabinoid acids undergo decarboxylation at the high temperatures necessary (200° to 250°C). For a routine analysis this may be an advantage, for this reaction parallels the smoking process. Gas-liquid chromatographic analysis will thus give directly all the THC available to a smoker in a certain sample. When an exact determination of the content is required, decarboxylation can be prevented by esterification.

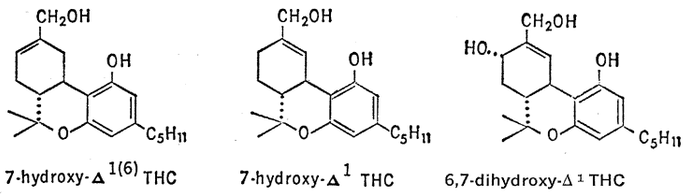


Fig. 8. Tetrahydrocannabidiol metabolites.

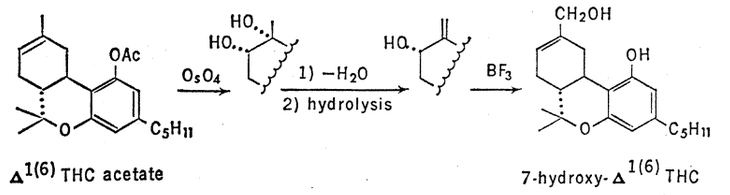


Fig. 9. Synthesis of the major $\Delta^{1(6)}$THC metabolite.

Metabolism

Cannabis sativa grown in an atmosphere of $^{14}CO_2$ yields generally labeled cannabinoids. Carbon-14-labeled Δ^1THC has been isolated from the cannabinoid mixture by thin-layer chromatography. When this labeled compound was administered intraperitoneally to a rat, 68 percent of the radioactivity was eliminated in the feces, and 12 percent via the urine within 5 days (*65*). The products of the biotransformation were unidentified polar compounds.

Table 1. Retention times on gas-liquid chromatography and R_F values on thin-layer chromatography of some neutral natural cannabinoids.

Compound	R_F*	Retention time†
Cannabicyclol	0.62	4′33″
Cannabidiol	.58	5′40″
$\Delta^{1(6)}$THC	.57	7′10″
Δ^1THC	.51	7′52″
Cannabinol	.47	10′12″
Cannabichromene	.43	5′35″
Cannabigerol	.42	9′20″

* Chromatoplates of silica gel. Elution with petroleum ether and ether in a ratio of 4 : 1. † Column: OV17 on Gas Chrom Q; N_2 flow: 30 cm³/min; column temperature, 235°C.

The $\Delta^{1(6)}$THC isomer has been specifically tritiated at the C-2 position by the isomerization of Δ^1THC with tritiated *p*-toluenesulfonic acid (*66*). This material was administered intravenously to rabbits. Examination of the urine indicated that several water-soluble substances containing tritium were present. These "conjugates" are not glucuronides; they are partially soluble in ether. Treatment of the mixture with 0.1*N* perchloric acid in ethyl acetate at 50°C gave one major compound (*67*). The same metabolite was obtained by incubation of tritiated $\Delta^{1(6)}$THC with a homogenate of rabbit liver (*68*). Examination of this metabolite by mass spectrometry and by some chemical transformations showed that a hydroxyl group had been introduced in the terpenoid moiety of $\Delta^{1(6)}$THC in an allylic position to the double bond. Synthesis studies indicated that the metabolite is not 2α- or 2β-hydroxy-$\Delta^{1(6)}$THC and the structure of the metabolite was tentatively put forward as 7-hydroxy-$\Delta^{1(6)}$THC (Fig. 8). On being boiled with acid, this metabolite is converted into cannabinol (on thin-layer chromatography data) (*67*). The suggested structure of the metabolite has been confirmed by synthesis (Fig. 9) (*68*). Synthetic 7-hydroxy-$\Delta^{1(6)}$THC when administered to monkeys is active at approximately the same dose level as $\Delta^{1(6)}$THC (*24, 68*).

Labeling of the aromatic hydrogens in Δ^1THC has been accomplished by heating Δ^1THC with tritiated water, phosphoric acid, and tetrahydrofuran

(*69*). The same investigators also reported the synthesis of Δ^1THC specifically labeled with ^{14}C at C-7 (*38*). Intravenously injected tritiated Δ^1THC is eliminated very slowly by the rat; half of the administered dose remains in the body after 1 week. About 80 percent is excreted in metabolized form via the feces, the remainder being eliminated, also as metabolites, in the urine. The metabolized THC is not excreted as a glucuronide, but may be partly protein bound. A considerable amount (17 to 40 percent) of the radioactivity excreted in the urine during the first 24 hours can be extracted with ether (*69*).

In the rabbit, the metabolism of THC is somewhat different. Tritiated Δ^1THC is excreted in the form of polar metabolites, mainly in the urine. About 35 percent of the radioactivity is eliminated via this route during the first 24 hours and only 10 percent in the feces (*70*). The distribution of radioactivity in tissues largely reflects the elimination through liver and kidney. Surprisingly, of all investigated tissues the spinal cord and brain show the least radioactivity. Tritiated Δ^1THC in the rabbit is metabolized at a fast rate, the half-life period in the blood after intravenous injection being 7 to 16 minutes. The amount of ether-soluble metabolites in the blood reaches a maximum between ½ to 2 hours after injection (*70*).

Liver homogenate or the supernatant of liver homogenate metabolized Δ^1THC to the psychopharmacologically active 7-hydroxy-Δ^1THC, which was identified by mass spectrometry and nuclear-magnetic-resonance spectroscopy (*71*). An inactive metabolite, 6,7-dihydroxy-Δ^1-THC, was also identified.

One report indicated that, in the rat, 44 percent of intraperitoneally administered THC was excreted via the bile as a conjugate of unchanged THC (*72*). The doses injected, however, seem to be very high and may have no relevance to actual THC consumption. Radioactivity has been found in the placenta and fetus of the pregnant Syrian hamster after intraperitoneal or subcutaneous administration of tritiated Δ^1THC. Although the absolute amounts of Δ^1THC reaching these organs were not stated, the unchanged compound was indicated to be responsible for 55 and 25 percent of the activity present in the placenta and fetus, respectively (*73*).

Facile deuteration and tritiation of both Δ^1THC and $\Delta^{1(6)}$THC by the use of labeled trifluoroacetic acid has been reported (*74*).

Little metabolic work in humans has been published. Cannabidiol—but neither Δ^1THC nor cannabinol—was identified in urine (after enzyme treatment) of a subject who had drunk tea with marihuana (*75*).

In the absence of definite evidence, one can speculate that a metabolite and not Δ^1THC is in fact the active compound on the molecular level. Habitués say that marihuana has no effect when used for the first time. While the basis of their observations may be psychological, it is also possible that it is due to a biochemical phenomenon. If the hydroxylation enzyme were an inducive one, then the initial administration of marihuana may be the triggering act for its formation. Subsequent THC introduction in the body will lead to rapid formation of the active hydroxy-THC. Some scattered data tend to support this conjecture. Both Δ^1THC and $\Delta^{1(6)}$THC are more active when administered intraperitoneally rather than subcutaneously. It has been suggested (*11*) that this may be due to the formation of active metabolites on passing through the liver. There seems to be a rough correlation between the rate of formation of the metabolite or metabolites in the rabbit (*70*) and the generally known, fast action of marihuana in humans. Though numerous critical criteria have yet to be satisfied before the acceptance of an active metabolite theory, as a working hypothesis it can stimulate work in several directions.

This short review should make it obvious that cannabinoid research has reached a stage where, after the chemical foundation has been laid, rapid and important progress in the biological, psychological, and clinical aspects is to be expected.

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Phage, Colicins, and Macroregulatory Phenomena

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The early work on bacteriophage growth, mutation, and recombination had the good fortune to serve as one avenue in the growth of molecular biology to its present state of an intellectually satisfying construction. It is unnecessary to recount today the story of that early work, in which it was my fortune to be engaged in friendly and exciting cooperation with Max Delbrück and Alfred Hershey. Even more difficult would be an attempt to trace here the series of developments that led from early phage work to the modern knowledge of virus reproduction, gene replication, and gene function and its regulation. My greatest satisfaction derives from the role that has been played by my students and co-workers in these developments and from the personal experience of association with many of the protagonists of this great intellectual adventure.

Phage research has branched off in many directions, each of which has contributed in some measure to the edifice of molecular biology. One of the most notable directions was that of gene function and its regulation. The major contributions of phage research in this area were made in the study of lysogeny by André Lwoff and François Jacob, which led to the formulation of the operon theory by François Jacob and Jacques Monod. The regulatory phenomena considered by this theory concerned the functions of individual genes or groups of genes. In this article I wish to deal with approaches to certain aspects of cellular regulation that involve "macroregulatory phenomena." By this I mean those phenomena in which the functional changes observed affect some of the major processes of the living cell, such as the syntheses of DNA, RNA, or protein; or the energy metabolism; or the selective permeability function of cellular membranes.

The study of antibiotics like penicillin or streptomycin, agents that act in a "molar" way on cellular processes, has played an important role in elucidating such processes as the organization of the biosynthesis of the bacterial cell wall or the mechanism of protein synthesis. When an alteration of a major cellular function is produced by the action of an agent such as a bacteriophage or some other macromolecular agent acting in a "quantal," single particle fashion, the situation is even more challenging since some mechanism of amplification must intervene between the individual unit agent and the affected elements of the responding cell. For a viral agent, the amplification mechanism may be the replication of the agent or the expression of its genetic potentials. For a protein agent, for example a bacteriocin, the amplification mechanism must be a change in the integrity of some cellular structure or of the functioning of some cellular control system. In either case, an understanding of the mode of action of such agents on major cellular processes is likely to reveal some interesting aspects of the functional organization of the cellular machinery.

In my laboratory, we are currently using bacteriophages and bacteriocins as

The author is professor of biology at the Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. This article is the lecture he delivered in Stockholm, Sweden, on 12 December 1969 when he received a Nobel Prize in Physiology or Medicine, which he shared with Dr. Max Delbrück and Dr. Alfred Hershey. It is published here with the permission of the Nobel Foundation and will also be included in the complete volume of *Les Prix Nobel en 1969* as well as in the series Nobel Lectures (in English) published by the Elsevier Publishing Company, Amsterdam and New York. The lectures of Dr. Delbrück and Dr. Hershey will be published in subsequent issues.