The Isolation and Structure of Δ1-Tetrahydrocannabinol and Other Neutral Cannabinoids from Hashish

Yehiel Gaoni and Raphael Mechoulam

Contribution from the Department of Chemistry, The Weizmann Institute of Science, Rehovoth, and the Laboratory of Natural Products, Hebrew University Pharmacy School, Jerusalem, Israel. Received April 27, 1970

Abstract: The isolation and elucidation of the structures of Δ1-tetrahydrocannabinol (Δ1-THC), cannabigerol, cannabichromene, and cannabicyclol are described. A facile conversion of cannabidiol into Δ1-THC takes place on treatment with boron trifluoride etherate. The absolute configuration of the chiral centers at C-3 and C-4 of Δ1-THC is established as R.

The resin of the female Cannabis sativa L. plant has been used as a medicine and a psychotomimetic drug since ancient times.1 Cannabis preparations were known to the Assyrians, Scythians, ancient Chinese, Indians, and Persians. More recently, increased consumption of either the resin (hashish) or the whole flowering top (marijuana) has caused worldwide social, legal, and medical problems.

The chemistry of the constituents of Cannabis has been the subject of numerous publications since the middle of the last century.2 Due mainly to the masterly investigations of Cahn,3 Adams,4 Bergel,5 and Todd6 substantial progress was made in this field. However, until 1963, when the structure of cannabidiol (Ia) was elucidated,7 the only cannabinoid with fully known structure was not fully known, and it was unavailable from either a natural or a synthetic source. For the reason the slow progress is to be found in the lack of suitable separative and analytical techniques in the thirties and early forties, when the important work in Urbana8 and Cambridge9 took place. As reproducible pharmacological and clinical investigations can only be undertaken with well-defined materials, this incomplete chemical evaluation of marijuana resulted in an almost total absence of fundamental experimental work on the biological aspects of the Cannabis problem. This lack of data on the pharmacological effects has had, in turn, serious social repercussions in the present wave of marijuana use.

In a number of communications8 we reported the isolation, structure elucidation, and absolute configuration of some neutral cannabinoids, including the major active constituent, Δ1-tetrahydrocannabinol (Δ1-THC). We wish to describe now the full details of this research.

Previous work2 indicated that the active constituent(s) were found in the petroleum ether extract of hashish. We were able to confirm and extend this observation. Benzene and methanol extracts from hashish, which had previously been repeatedly extracted with petroleum ether, were found to be inactive when tested in rhesus monkeys.9 Hence we concentrated on the petroleum ether fraction, which was separated into neutral and acidic components. The acid fraction was inactive.10 The following compounds and mixtures were isolated from the active, neutral fraction by repeated chromatography on Florisil or acid-washed alumina, and alumina containing 12% silver nitrate (in order of increasing polarity): (1) a mixture of waxy, noncannabinoid materials; (2) cannabicyclol (III); (3) cannabidiol (Ia);11 (4) Δ1-THC (IVA); (5) cannabiol (II); (6) cannabichromene (VA); (7) cannabigerol (VI); and (8) polar constituents and polymers. The yields of cannabinoids are indicated in Table I.

Table I. Content in Hashish.* Retention times (vpc) of Some Natural Neutral Cannabinoids

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yields*</th>
<th>Rf</th>
<th>Retention timec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabicyclol (III)</td>
<td>0.11</td>
<td>0.62</td>
<td>4' 33&quot;</td>
</tr>
<tr>
<td>Cannabidiol (Ia)</td>
<td>3.74 (1.4) (2.5)</td>
<td>0.58</td>
<td>5' 40&quot;</td>
</tr>
<tr>
<td>Δ1-THC (IVA)</td>
<td>3.30 (1.4) (3.4)</td>
<td>0.51</td>
<td>7' 52&quot;</td>
</tr>
<tr>
<td>Δ1-THC (VIII)</td>
<td>1.30 (0.3) (1.2)</td>
<td>0.47</td>
<td>10' 12&quot;</td>
</tr>
<tr>
<td>Cannabichromene (VA)</td>
<td>0.19</td>
<td>0.43</td>
<td>5' 35&quot;</td>
</tr>
<tr>
<td>Cannabigerol (VII)</td>
<td>0.30</td>
<td>0.42</td>
<td>9' 20&quot;</td>
</tr>
</tbody>
</table>

* As per cent of hashish; determined by vpc. The numbers in parentheses are from two partial analyses of different batches.

The active Δ1-THC represented 3.3% of the sample. Partial analyses of different batches showed the presence of 1–5% Δ1-THC. Cannabis preparations vary widely in their content of cannabinoids.10,11 This

(1) R. J. Bouquet Bull Narcotics, 3 (3), 22 (1951), and references cited therein, describes in fascinating detail the history of Cannabis use and the various preparations and modes of consumption in different parts of the world.


(4) R. Adams, Harvey Lect., 37, 168 (1942).


variability may be the result of many factors such as climate, soil, mode of preparation, length of storage, etc. In this connection it should be pointed out that on heating the inactive cannabinoid, acids are decarboxylated to yield the corresponding neutral cannabinoids. Hence for practical estimation of the Δ1-THC available on smoking, the amount of Δ1-THC acid A (IVb) and Δ1-THC acid B (IVc) should be taken into consideration. In hashish these acids constitute 1–3%.

The above figures are compatible with the popularly accepted notion that Middle Eastern hashish and Indian charas contain 5–6 times more active material than American marijuana.\(^\text{11}\)

\[\text{Ia, } R=H \quad \text{II, } b, R=COOH; R" = H\]

\[\text{III, } \text{IVa, } R'= R" = H \quad \text{Va, } R= H \quad \text{b, } R=COOH\]

\[\text{Va, } R= H \quad \text{b, } R=COOH\]

\[\text{VI, } \text{a, } R= H \quad \text{b, } R=COOH\]

\[\text{Va, } R= H \quad \text{b, } R=COOH\]

\[\text{VI, } \text{a, } R= H \quad \text{b, } R=COOH\]

\[\text{Va, } R= H \quad \text{b, } R=COOH\]

\[\text{VI, } \text{a, } R= H \quad \text{b, } R=COOH\]

\[\text{Va, } R= H \quad \text{b, } R=COOH\]

\[\text{VI, } \text{a, } R= H \quad \text{b, } R=COOH\]

\[\text{Va, } R= H \quad \text{b, } R=COOH\]

\[\text{VI, } \text{a, } R= H \quad \text{b, } R=COOH\]

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\[\text{VI, } \text{a, } R= H \quad \text{b, } R=COOH\]

\[\text{Va, } R= H \quad \text{b, } R=COOH\]

\[\text{VI, } \text{a, } R= H \quad \text{b, } R=COOH\]

\[\text{Va, } R= H \quad \text{b, } R=COOH\]

\[\text{VI, } \text{a, } R= H \quad \text{b, } R=COOH\]

\[\text{Va, } R= H \quad \text{b, } R=COOH\]

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\[\text{VI, } \text{a, } R= H \quad \text{b, } R=COOH\]

\[\text{Va, } R= H \quad \text{b, } R=COOH\]
In 1942 Wollner, et al.,\(^*\) isolated from marihuana an active product which could be converted into cannabinol (II). It was different from the synthetic semisynthetic THC's prepared by Adams. No definite structure was proposed for this material, though it was assumed to be a THC isomer. The \([\alpha]D -193^\circ\) reported for this substance indicates now that it was probably impure \(\Delta^1\)-THC \([\alpha]D -150^\circ\). A few additional reports on the isolation of active materials from Cannabis have appeared in the literature. Haagen-Smit, et al.,\(^{21}\) and Powell, et al.,\(^*\) have published short communications on the isolation of active materials. These reports lack details to allow comparison with later work. de Ropp\(^{11}\) has described the isolation of a THC. Its infrared spectrum and some other physical properties are similar to those of \(\Delta^1\)-THC. These reports deal mainly with the isolation of material and do not contribute any additional data as to the structure(s).

The fact that different THC's or mixtures of isomers showed activity led to the generally accepted belief that the activity of Cannabis was due to a mixture of isomers. This view is in our opinion not correct, the activity being due largely, or almost exclusively, to \(\Delta^1\)-THC. However two additional active compounds have been identified. Hively, et al.,\(^{1a}\) reported the presence of \(\Delta^{16}(8)-\)THC (VIII) in marihuana: the ratio of \(\Delta^{16}(8)-\)THC to \(\Delta^1\)-THC was ca. 1:10. Most Cannabis samples, which have been analyzed, however, contain considerably less \(\Delta^1\)-THC, the ratio being ca. 1:100 or even lower.\(^{11}\) The \(\Delta^1\) isomer is more labile than the \(\Delta^{16}(8)\) isomer; hence the ratio will vary also depending on the length and conditions of storage. Very recently an active homolog of \(\Delta^1\)-THC, to which Adams, et al.,\(^{*}\) have assigned structure XI, was isolated independently by Claussen, et al.,\(^{21}\) and Pu, et al.,\(^{22}\) in Pakistani hashish. It has been assigned structure XII, and is 4.8 times less active than \(\Delta^1\)-THC in its cataleptic activity in mice. This homolog does not seem to be present in hashish on the basis of vpc measurements.

In our preliminary communication\(^{8b}\) we reported that \(\Delta^1\)-THC showed strong ataxia activity in dogs. Full details of the animal tests have since been published.\(^{8b}\) Detailed human experiments were not undertaken by us, but on the basis of preliminary tests on volunteers we reported that the effective dose in humans was 3-5 mg.\(^{2}\) Later, numerous groups reported on the activity of \(\Delta^1\)-THC in animals and humans.\(^{23}\) However as yet our understanding of the molecular basis of THC action is negligible.

cannabinol (Ia) with p-toluenesulfonic acid, however, gives essentially one \(\Delta^1\)-THC isomer, \(\Delta^1\)-THC, to which Adams, et al.,\(^{13}\) have assigned structure VIII (without stereochemistry).\(^{21}\) \(\Delta^1\)-THC (IVa) was not isolated in pure form either from a natural material or from a semisynthetic mixture by the groups in Urbana or Cambridge.\(^{24}\)


Cannabinol (VI). This minor component is the only known neutral cannabinoid whose stage of oxidation is lower than that of the rest of the group. We have assumed\(^{26}\) that cannabinol is formed in nature from geraniol and olivetol and hence represents the initial product of cannabinoid biogenesis.

Cannabinol has two reducible double bonds (as determined by microhydrogenation). The uv spectrum of cannabinol shows the absence of conjugation. The nmr spectrum indicates that (a) the two aromatic hydrogen atoms are magnetically equivalent, (b) the protons of the methylene group at C-8 are strongly deshielded, and split by a single adjacent proton, which is presumably due to the \(\Delta^2\) double bond, and (c) three olefinic methyl groups are present, which suggests that the second double bond is at the \(\Delta^2\) position. Assuming that the side chain is of the normal terpenoid type these findings are compatible with structure VI only.

The structure of cannabinol has been confirmed by syntheses.\(^{26,25}\)

Cannabichromene (Va). This minor component was isolated independently by Claussen, et al.,\(^{27}\) and by us\(^{26}\) and through a coincidence was given the same name by both groups. The \([\alpha]D\) was reported as +3.4 and −9°. Later work\(^{26}\) has indicated that when the oily cannabichromene is further purified, no rotation is observed. A crystalline derivative, a 3,5-dinitrophenylurethane, mp 106–107°, has likewise no rotation. Cannabichromene (Va) has been correlated with cannabinol (Ia) via \(\Delta^{11,12}\)-THC (XII).\(^{16}\) This compound when obtained from cannabinol has a rotation of \([\alpha]D -300°\), while when prepared from

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cannabinichromene it shows no rotation.\textsuperscript{16} We assume therefore that natural cannabichromene is indeed racemic. The related cannabichromenic acid (Vb) originally reported\textsuperscript{29a} to be optically active is in fact probably also racemic.\textsuperscript{29b} This lack of optical activity points out that either (a) cannabichromene is an artifact, apparently formed by nonenzymic oxidation of cannabigerol, or that (b) the intermediate formed by enzymic oxidation is a symmetrical species such as XIV.

The uv spectrum of cannabichromene indicates conjugation of one of the double bonds with the ring and is compatible with the spectra of similar chromenes derived from resorcinol derivatives.\textsuperscript{29} The nmr spectrum indicates that (a) the two aromatic protons are magnetically nonequivalent and that one of the methyl groups on the terpene moiety is \( \alpha \) to an oxygen atom thus determining the point of attachment of the ether–oxygen atom, the other oxygen atom being in a free phenolic group; (b) two of the olefinic protons are not flanked by additional hydrogen atoms (sharp AB pattern, \( \delta 5.44, 6.60, J_{AB} = 10 \text{ Hz} \)); (c) the second double bond is in an isopropylidene grouping (two methyl groups, \( \delta 1.58, 1.62 \), one olefinic proton, \( \delta 5.05 \)). These findings are compatible only with structure Va.

Cannabichromene has been correlated with cannabigerol by hydrogenation to the oily tetrahydrocannabinol (XV), the 3,5-dinitrophenylurethane of which melts at 127–128°. The same tetrahydrocannabinol (XV) (3,5-dinitrophenylurethane, mp 127–128°) was obtained from cannabigerol by boiling with \( \beta \)-toluenesulfonic acid in benzene to give a mixture of XVI\textsuperscript{28} and XVII; reduction of XVII gave XV. Pure cannabichromene shows no activity in the dog ataxia or monkey behavioral tests in doses up to 10 mg/kg.\textsuperscript{30b} The positive dog ataxia test previously observed\textsuperscript{28} was probably due to impurities in the natural material, which was available in minute amounts.

The structure of cannabichromene has been confirmed by syntheses.\textsuperscript{30}

Cannabicyclol (III). This minor component, mp 46°, was first isolated by Korte and Sieper.\textsuperscript{31} It was initially\textsuperscript{31} considered to have a THC-type structure and was named “THC III.” It was later independently isolated by our group\textsuperscript{3} and renamed cannabicyclol. The molecular weight (mass spectrum) and elementary analysis indicated the composition \( C_{20}H_{20}O_2 \). The uv spectrum is typical for the olivetol moiety. The nmr spectrum shows (a) two nonequivalent aromatic protons, (b) four methyl groups, none of which is olefinic, but at least one is \( \alpha \) to an oxygen atom and one is apparently the terminal methyl group of the pentyl side chain, and (c) no olefinic protons. Apparently cannabicyclol has no double bonds. Consequently, the elemental composition requires a tetracyclic structure. Structure XVIII was suggested as a working hypothesis.\textsuperscript{2} Parallel to our work, Claussen, et al.,\textsuperscript{32} put forward XVIII as a definite structure for the same compound (now renamed cannabipinol). From a synthetic sequence Crombie and Ponsford\textsuperscript{33} isolated a material which was shown to be identical with cannabicyclol and to which the correct structure was assigned. A 220-MHz nmr spectrum shows that the benzyl C-3 proton is a doublet being coupled to the C-2 proton only. The only structure which fits these data is III.

The suggested constitution of cannabicyclol has received further support from an unequivocal photochemical synthesis.\textsuperscript{23} Other syntheses have also been achieved.\textsuperscript{30b,c,34}

Cannabicyclol shows no rotation. In view of its presumed formation from cannabichromene\textsuperscript{33} this

\begin{align*}
&\text{(28) (a) Y. Shoyama, T. Fujita, T. Yamauchi, and I. Nishioka,} \\
&\text{Chem. Pharm. Bull., 16, 1157 (1968); (b) T. Yamauchi, private} \\
&\text{communication.}\n\end{align*}

\begin{align*}
&\text{(29) H. Fukami, M. Nahayama, and M. Nakajima,} \\
&\text{J. Chem. Soc., 1121 (1940); G. Cardillo, L. Merlini, and R. Mondelli,} \\
&\text{Tetrahedron, 24, 497 (1968).}\n\end{align*}

\begin{align*}
&\text{(30) (a) R. Mechoulam, B. Yagnitsinsky, and Y. Gaoni,} \\
&\text{J. Amer. Chem. Soc., 90, 2418 (1968); (b) L. Crombie and R. Ponsford,} \\
&\text{Chem. Commun., 894 (1968); (c) V. V. Kane and R. K. Razdan,} \\
&\text{J. Amer. Chem. Soc., 90, 6551 (1968); (d) G. Cardillo, R. Cricchio, and L. Merlini,} \\
&\text{Tetrahedron, 24, 4825 (1968).}\n\end{align*}

\begin{align*}
&\text{(31) F. Korte and H. Sleper,} \\
&\text{J. Chromatogr., 13, 90 (1964).}\n\end{align*}

\begin{align*}
&\text{(32) U. Claussen, F. von Spulak, and F. Korte,} \\
&\text{Tetrahedron, 24, 1021 (1968).}\n\end{align*}

\begin{align*}
&\text{(33) L. Crombie, R. Ponsford, A. Shani, B. Yagnitsinsky, and R.} \\
&\text{Mechoulam,} \\
&\text{Tetrahedron Lett., 5771 (1968).}\n\end{align*}

\begin{align*}
&\text{(34) B. Yagen and R. Mechoulam, ibid., 5153 (1969).}\n\end{align*}
is not surprising. It remains to be established whether it is formed in the plant (possibly via a photochemical process from Va) or is formed in the resin on storage. It cannot be an artifact of the isolation and purification procedures as these are mild and do not involve steps conductive to cannabinomere cyclization.

**Absolute Configuration.** Adams, *et al.*, have reported that tetrahydrocannabinol (XIX) obtained by reduction of cannabinol (which has since been shown  to possess structure Ia) can be oxidized to the menthane carboxylic acid XXa. The anilide of XXa thus obtained did not depress the melting point of the anilide of XXa prepared from menthol (XXI) through the menthyl chloride (XXII), followed by carbonation of the Grignard derivative. However, the rotation of the anilide of XXa prepared by the degradation of the natural product was not reported.

We have repeated and extended this correlation. Catalytic hydrogenation of natural (−)-cannabinol gave a mixture of the two C-1 epimers (XIX) which could be separated by column chromatography on Florisil. The chromatographically more polar isomer was oxidized with potassium permanganate in acetone. The acidic product obtained was esterified with diazomethane and purified by preparative vapor phase chromatography. The pure menthane carboxylic acid methyl ester (XXb) thus obtained ([α]D = −20°) was identical in all respects (ir, nmr, tlc, rotation) with XXb prepared from natural (−)-menthol (XXI) through the acid XXa, followed by methylation. Basic hydrolysis of XXb, obtained by degradation of cannabinol, gave menthane carboxylic acid (mp 64–65°, [α]D = −44°) also identical in all respects (ir, nmr, tlc, [α]D, mixture melting point) with XXa prepared from menthol (XXI).

Natural (−)-menthol has been interrelated with glycerinaldehyde. This correlation establishes therefore the absolute configuration of cannabinol (Ia). As the latter has been converted into Δ^+THC (IVa), and into Δ^{10H}-THC (VIII), the above correlation establishes the absolute configuration of these natural products at both C-3 and C-5, as R. Šantávy has reached similar conclusions, mainly by comparison of optical rotation data from the literature. However, some of the rotations compared were of compounds which were later shown to be mixtures.

These absolute configurations were later confirmed by total syntheses which started from optically active terpenes with known chirality.

**Analytical Aspects.** In view of the practical importance of *Cannabis* analysis, numerous groups have investigated this problem. Thin layer chromatography methods are widely used for qualitative analysis. A popular procedure is the one suggested by Korte and Sieper. It employs silica gel impregnated with dimethylformamide (DMF); cyclohexane is used as eluent. It has been reported, however, that the Rf values in this system are affected by the grade of dryness of the DMF.

Numerous other solvents have also been employed. In our investigations we have used a rather simple system: chromatoplates of silica gel; elution with petroleum ether (bp 40–60°)–ether in a ratio of 8:2. The plates were sprayed with a potassium permanganate solution. The Rf values of the major natural neutral cannabinoids are tabulated in Table I. Vapor phase chromatography has been extensively employed. The columns in use today are SE 30, XE 60, Carbowax 20 M, OV 17, OV 1. We have routinely used 2% OV-17 on Chromosorb Q at 235°. The retention times of the major natural neutral cannabinoids are tabulated in Table I. It should be pointed out that all cannabinoid acids undergo decarboxylation at the high temperatures employed for vpc (200–250°). For a routine analysis this may be an advantage, for this reaction parallels the smoking process. A vpc analysis will thus give directly all the THC available on smoking in a certain sample. When an exact determination of the content is required, decarboxylation can be prevented by esterification.

**Experimental Section**

**General.** The ir spectra were recorded on a Perkin-Elmer Model 137 instrument, the nmr spectra were measured on a Varian A-60 spectrometer, and the uv spectra were measured on a Cary 14 spectrometer. The ir curves of the natural neutral cannabinoids described in this paper have been reproduced. Detailed uv and nmr spectra of these compounds have been described. Most of the mass spectra have been reported. The remaining mass spectra were measured on an Atlas CH4 instrument: tlc, chromatoplates of silica gel G (Merck), elution with petroleum ether (bp 40–60°) and ether in a ratio of 4:1; developer, 0.3% potassium permanganate in a saturated solution of cupric acetate. Vapor phase chromatography was conducted on a Packard Model 803 with a flame ionization detector, glass columns (6 ft × 1/8 in.) with 2% OV-17 on Gas Chrom Q. N, flow rate 30 cm/min, column temperature 235°. The microanalyses were performed by the microanalytical department of the Weizmann Institute.

**References**


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Extraction of Hashish. Hashish "soles" of undetermined age (not less than 1 year old) were obtained from police sources. They originated from various "producers," presumably in Lebanon, as indicated by the stamped markings on their cloth covers. Each weighed ca. 200 g. Two "soles" (402 g) were broken into small pieces and stirred mechanically with 4 l of petroleum ether (b.p. 60-80°) in a glass vessel. The mixture was filtered with suction, the extraction was repeated twice, and the extracts were combined. A total of 135 g of extract was obtained, as determined by evaporating an aliquot to dryness under high vacuum and weighing the dry oil. The brown petroleum ether solution was concentrated to ca. 1 l, and was then extracted three times with 250 ml of an aqueous solution of 5 % sodium hydroxide and 2% sodium sulfite in the presence of ice. The aqueous phase was worked up as described before to yield 28 g of acids (6.96% with 250 ml of an aqueous solution of 5% sodium hydroxide and 2% sodium sulfite in the presence of ice). The dark brown petroleum ether solution was then washed with a saturated solution of sodium chloride (200 ml), dried over sodium sulfate, and evaporated to give 65 g (16.2% hashish) of a dark viscous oil. In addition to the two liquid phases a dark tar (21 g, 3-6% by weight) of silver nitrate was obtained from the latter by chromatography on alumina coated with 3,5-dinitrobenzoyl chloride in pyridine, followed by chromatography on silica gel (elution with 2% ether in pentane). This yielded a homogenous product by tic, which could not be induced to crystallize; [αD]275 = -71°.

Analysis. For CdH24NO6: C, 64.23; H, 6.35. Found: C, 64.17; H, 6.54.

The second solid (0.4 g) was recrystallized from hexane and identified as the amide VIIb: mp 145-146°; δ (CCl4) 0.9, 1.04, 1.38 (-CH3 groups), 3.12 (br d, C-3 H), 6.05 (d, aromatic H), 6.18 (d, aromatic H), 6.20 (d, aromatic H). This yielded the hydrochloride, mp 145-146°; δ (CDCl3) 0.88 (3H, aromatic H), 10.4 (hydrogen bonded OH, exchangeable with D2O). Anal. Calcd for CdH24NO6·HCl: C, 64.45; H, 6.61.

Hydrolysis of the 3,5-Dinitrophenylurethane of Δ-THC (VIIa) to Δ-THC. The urethane (300 mg) was hydrolyzed in ethanol, a 1% excess of the catalyst) by boiling with 10% hydrochloric acid solution of potassium hydroxide solution was added, and the solution was warmed in a water bath at 60-65° for 10 min. Water was added to the cooled solution which was then extracted with pentane. The pentane extract was washed with water and dried over sodium sulfate. Evaporation of the pentane and redissolution in a small amount of the solvent left some insoluble dinitroaline, which was filtered. Chromatography of the pentane solution on alumina yielded pure Δ-THC. It was distilled in a bulb-to-bulb distillation apparatus, bp ca. 220° (bath temperature) (0.1 mm). This compound did not differ by any of the standard criteria of purity from the material as obtained after repeated chromatography (fr, uv, nmr, tic, [αD]275). Anal. Calcd for CdH24NO6: C, 80.21; H, 9.55. Found: C, 80.20; H, 9.55.

Dehydrogenation of Δ-THC (IVA) to Cannabinol (II). A mixture of 170 mg of Δ-THC (IVa) and 40 mg of sulfur was heated at ca. 250° for 45 min. It was dissolved in benzene and chromatographed on a small column of silica gel. The oil was distilled in a bulb-to-bulb apparatus, bp ca. 200° (bath temperature) (0.1 mm). The compound obtained was identical with cannabinol isolated from hashish (fr, nmr, tic, vpc). The acetate of II, mp 76-77°, from dehydrogenation, did not depress the mp of II, 76-77°, from hashish.
Cannabinol (II) from Hashish. This constituent \(^{48}\) was obtained as described above by chromatography of crude fractions containing \(\Delta^2\)-THC and cannabiniol. While it is possible to obtain crystalline II, mp 75–76\(^{\circ}\), it is more easily identified as the acetate, mp 76–77\(^{\circ}\).

A 3,5-dinitrophenylurethane of II was prepared as described above by evaporation of 223–234\(^{\circ}\) (hexane-ether).

\[ \text{mp 75-76}^{\circ}, \text{it is more easily identified as the acetate, mp 76-77}^{\circ}. \]

The more polar fraction (1.2 g) was identified as XIX, without further purification. Potassium permanganate (2.5 g) was added to the oil, which was oxidized to the corresponding aldehyde. The residue had a strong fatty acid smell. It was treated with excess diazomethane. The oily ester was purified by preparative vpc through the Grignard derivative followed by carbonation to XXa via menthyl chloride (XXII), \(^{36}\). The ester (XXb) and the acid (XXa) were identical in all respects with the natural \(\Delta^2\)-THC (IVa).

\[ \text{Anal. Calcd for C}_{30}H_{40}O_{7}: C, 64.12; H, 6.71; N, 8.15.} \]

Tetrahydrocannabinol (XV) was prepared by catalytic hydrogenation of as described for the reduction of cannabinol to XV (at 150° on a 0.2% Apiezon L on glass beads). The pure menthane derivative was obtained. Potassium permanganate (2.5 g) was added to a solution of XV in ethanol (20 ml) with platinum black as catalyst. After 1 hr the catalyst was filtered off, the solvent was evaporated, and, as the oil obtained still showed the presence of an olefinic proton in the nmr, the reduction was repeated. The product was chromatographed on 150 g of Florisil. Elution with 2\% ether in pentane yielded two fractions. The less polar one (600 mg) was shown by vpc to be a mixture. The nmr spectrum of this mixture showed no olefinic protons or methyls, indicating that it probably consists of the two C-1 epimers of tetrahydrocannabinol. The more polar fraction (1.2 g) was identified as XIX, on the basis of its nmr spectrum, \(\delta (\text{CCl}_3) 0.75, 0.88 (\text{CH}_3\text{ groups}), 6.0, 6.12 (2 \text{ aromatic H}), \text{mol wt (mass spectrum) 316}. \)

\[ \text{Anal. Calcd for C}_{30}H_{40}O_{7}: C, 79.70; H, 10.19. \]

The 3,5-dinitrophenylurethane of XVII melts at 130–131\(^{\circ}\) and then at 146–147\(^{\circ}\) (benzene–pentane).

\[ \text{Anal. Calcd for C}_{30}H_{40}O_{7}N_2: C, 63.99; H, 6.71; N, 7.99.} \]

Elution with pentane–ether (92:2) gave 0.65 g of dihydrocannabinol (XVII), \(\Delta^8\)-Cannabichromene, \(\Delta^8\). It was clearly distinguishable from the cis isomer. The trans isomer XVI has \(\delta (\text{CCL}_3) 0.95, 1.05, 1.25 (4 \text{ CH}_3\text{ groups}), 6.10, 6.20 (2 \text{ aromatic protons}); \text{mol wt (mass spectrum) 316}. \)

\[ \text{Anal. Calcd for C}_{30}H_{40}O_{7}: C, 79.70; H, 10.19. \]

Tetrahydrocannabinol (XV) was prepared from XVII by catalytic hydrogenation as described for the reduction of cannabinol to XV. The nmr, ir, and uv spectra as well as the vpc and tlc behavior of XV from both reactions are identical. The 3,5-dinitrophenylurethane, mp 127–128\(^{\circ}\), of XV prepared from XV does not depress the melting point of XV prepared from cannabichromene.

Conversion of Cannabinol into Menthylcarboxylic Acid (XXa). Tetrahydrocannabinol (XIX) was obtained by hydrogenation of cannabidiol (Ia) (3 g) in ethanol (20 ml) with platinum black as catalyst. After 1 hr the catalyst was filtered off, the solvent was evaporated, and, as the oil obtained still showed the presence of an olefinic proton in the nmr, the reduction was repeated. The product was chromatographed on 150 g of Florisil. Elution with 2\% ether in pentane yielded two fractions. The less polar one (600 mg) was shown by vpc to be a mixture. The nmr spectrum of this mixture showed no olefinic protons or methyls, indicating that it probably consists of the two C-1 epimers of tetrahydrocannabinol. The more polar fraction (1.2 g) was identified as XIX, on the basis of its nmr spectrum, \(\delta (\text{CCL}_3) 0.75, 0.88 (\text{CH}_3\text{ groups}), 6.0, 6.12 (2 \text{ aromatic H}), \text{mol wt (mass spectrum) 316}. \)

\[ \text{Anal. Calcd for C}_{30}H_{40}O_{7}: C, 79.70; H, 10.19. \]

The 3,5-dinitrophenylurethane of XVII melts at 97\(^{\circ}\) (cyclohexane–pentane).

\[ \text{Anal. Calcd for C}_{30}H_{40}O_{7}: C, 63.99; H, 6.71; N, 7.99.} \]

Elution with pentane–ether (92:2) gave 0.65 g of dihydrocannabinol (XVII), \(\Delta^8\)-Cannabichromene, \(\Delta^8\). It was clearly distinguishable from the cis isomer. The trans isomer XVI has \(\delta (\text{CCL}_3) 0.95, 1.05, 1.25 (4 \text{ CH}_3\text{ groups}), 6.10, 6.20 (2 \text{ aromatic protons}); \text{mol wt (mass spectrum) 316}. \)

\[ \text{Anal. Calcd for C}_{30}H_{40}O_{7}: C, 79.70; H, 10.19. \]

Tetrahydrocannabinol (XV) was prepared from XVII by catalytic hydrogenation as described for the reduction of cannabinol to XV. The nmr, ir, and uv spectra as well as the vpc and tlc behavior of XV from both reactions are identical. The 3,5-dinitrophenylurethane, mp 127–128\(^{\circ}\), of XV prepared from XV does not depress the melting point of XV prepared from cannabichromene.
and methylation to XXb. The relative and absolute configurations of these compounds have been established.

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Studies of the Chymotrypsinogen A Family of Proteins. VIII.
Thermodynamic Analysis of Transition I of the Methionine Sulfoxide Derivatives of α-Chymotrypsin

Rodney Biltonen and Rufus Lumry*

Contribution from the Laboratory for Biophysical Chemistry, Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455. Received August 15, 1969

Abstract: Spectral changes at 293 nm have been used to monitor the first thermal-unfolding transition (transition I) of the monomethionine sulfoxide and dimethionine sulfoxide derivatives of α-chymotrypsin. From these data $\Delta F^0$, $\Delta H^0$, and $\Delta S^0$ have been calculated as a function of pH and temperature. Monomethionine sulfoxide chymotrypsin and its parent, chymotrypsin, show identical transition I characteristics. On the other hand, dimethionine sulfoxide chymotrypsin is thermodynamically less stable than its parent although transition I still exhibits all-or-none cooperativity. The thermodynamic results are in complete accord with the predictions of Brandts' ‘force’ analysis of protein unfolding and provide strong support for this type of analysis. For example, dimethionine sulfoxide chymotrypsin exhibits a temperature of maximum stability which is a characteristic consequence of a change in the number of interactions between water and the nonpolar moieties of the protein which occurs on unfolding. Parameters of Brandts' analysis of transition I evaluated with the aid of model compound data allow comparisons among α-chymotrypsin, chymotrypsinogen, and dimethionine sulfoxide α-chymotrypsin to be made. The cooperative unfolding units of dimethionine sulfoxide α-chymotrypsin and chymotrypsinogen are approximately one-half that of α-chymotrypsin. The results are consistent with results obtained by other investigators using nuclear magnetic resonance line widths as a measure of segmental flexibility and calorimetric measurements of enthalpy changes and heat capacity. It appears that the thermally unfolded states of all the chymotrypsinogen proteins thus far studied are very similar, although a significant amount of folded structure is retained in this state. Since the cooperative unfolding unit of dimethionine sulfoxide chymotrypsin is only about half that of its parent, this protein must be partially unfolded in its best folded state. The change in enzymic efficiency of dimethionine sulfoxide chymotrypsin may be related to this partial unfolding which apparently must be restored before chemical catalysis can take place.

* To whom correspondence should be addressed.

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