CONSTITUENTS OF *CANNABIS SATIVA*. XIX. ISOLATION AND STRUCTURE ELUCIDATION OF CANNABIGLENDOL, A NOVEL CANNABINOID FROM AN INDIAN VARIANT

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ABSTRACT.—An investigation of a hexane soluble fraction from the leaves and small stems of *Cannabis sativa* L. obtained from an Indian variant grown in Mississippi resulted in the isolation and characterization of cannabiglendol (8-hydroxy-isohexahydrocannabivarin). The structure of this compound was determined by spectral means and by examination of the ev-mf graphs of both the natural and the synthetic C_5-homolog.

Cannabis sativa L. and its preparations are the most widely used drugs of abuse. Investigations of *Cannabis* have resulted in the isolation and/or detection of more than 400 compounds (1). Cannabinoids, of which 60 are known, represent the most characteristic group of compounds in this plant.

In previous communications, we have reported the isolation and characterization of several hydroxylated cannabinoids with tetrahydrocannabinol structure type (2, 3, 4). In this communication, we report the isolation and characterization of a novel hydroxylated cannabinoid.

Gas chromatographic analysis of a relatively polar fraction of the leaves of an Indian variant of *Cannabis sativa* L. on 2% OV-17 showed a peak with the same relative retention time as that of Δ^9-THC. However, on tlc, this material was much more polar than Δ^9-THC. Spectral analysis (gc-ms) of this fraction showed a molecular ion at *m/e* 304. Repeated chromatography of this fraction resulted in the isolation of cannabiglendol (**1**) as a pale yellow oil.

EXPERIMENTAL[1]

PLANT MATERIAL.—The leaves and small stems of *Cannabis sativa* L. grown in Mississippi from seeds of an Indian variant (IN-B)[2] were used in this study. A voucher specimen has been deposited in the Herbarium, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi.

ISOLATION PROCEDURE.—The dried plant material (1 kg) was extracted with ethanol, and the concentrated extract was then washed several times with hexane. The hexane soluble material was chromatographed on a column of silica gel impregnated with silver nitrate and eluted with 80% petroleum ether in hexane. Fraction 8 (280 mg) from the above column was further chromatographed on a silica gel column. Fractions 1 and 2 eluted with hexane (50 ml)

[1]The ir spectra were obtained on a Beckman IR-33 or a Perkin-Elmer 257 recording spectrophotometer. Proton nmr spectra were obtained in deuterated chloroform on a JEOL C-60 HL or a JEOL Fx 60 spectrometer with tetramethylsilane as internal standard. Gc-ms analyses were carried out on a Varian 2740 gas chromatograph (Varian Instruments, Palo Alto, CA, U.S.A.) interfaced to a Dupont 491 mass spectrometer (Dupont Company Instrument Products, Wilmington, DE, U.S.A.). Helium was used as the carrier gas at a flow rate of 10–30 ml/min. The detector and inlet temperatures were 260° and 240°, respectively. Gc analysis: analyses were performed on Beckman GC-45 and GC-72-5 gas chromatographs equipped with flame ionization detectors and operated isothermally at 204°. The inlet temperature was 240°, and the detector temperature was 260°. Glass columns (0.63 cm 0.25 in) o.d.; 2 mm i.d. x 2.4 m (6 ft) were packed with 2% OV-17 or 6% OV-1 on 100–120 mesh chromosorb Q. Nitrogen was used as the carrier gas at a flow rate of 18–20 ml/min, depending on which instrument was used.

[2]Seed obtained for Dr. C. K. Atal of the Regional Research Laboratory, Jammu Tawi, India.

and hexane-ether (9:1, 200 ml), respectively, were combined. The solvent was evaporated to dryness, and the residue was triturated with ethanol and filtered. The filtrate, after evaporation to dryness, yielded a brown oil (115 mg). Column chromatography, followed by preparative tlc with hexane-ether (8:2) as the solvent system afforded 5.4 mg of a pure oily material as indicated by tlc and glc. On glc, this material had the same retention time (RRT 0.49) as Δ^9-THC using 2% OV-17. On tlc, the material showed one spot with R_f value 0.06 in hexane-ether (8:2) and acquired a violet coloration with Fast Blue B versus red for Δ^9-THC; ir max ($CHCl_3$) cm^{-1}: 3220 (sh), 3150, 1640, and 1580; ^{1}Hnmr ($CDCl_3$): δ6.47, δ6.38 (1H each, s, aromatic protons), δ3.28 (1H, br, for benzylic proton on C-3), δ2.54 (2H, t, benzylic protons of side chain), δ1.50 (6H, s), δ1.30 (3H, s, methyl on oxygenated carbon) and 0.95 (3H, t, for terminal methyl of side chain), ms: M^+ 304 (11%) and other ions at *m/e* 286 (13%), 271 (9%), 243 (15%), and 203 (100%).

A. SYNTHESIS OF 9-HYDROXY-HEXAHYDROCANNABINOL (9-OH-HHC)

1. *Preparation of Δ^9-THC acetate* (**X**).—To 500 mg of Δ^9-THC (**IX**) was added 2 ml of acetic anhydride and 1 ml pyridine; the reaction mixture was left overnight. The latter was poured on crushed ice and extracted with chloroform (3 x 10 ml). The combined chloroform extracts were washed with cold 10% H_2SO_4 followed by $NaHCO_3$ and water, and then dried over anhydrous magnesium sulfate. After evaporation of the solvent to dryness, a light orange-colored oil was obtained (**X**) (463 mg). This material was shown to be 96% pure by glc with 2% OV-17 (RRT 0.40). It gave the following data: gc-ms M^+ 356; ^{1}Hnmr ($CDCl_3$) δ6.50 and δ6.63 and δ6.10 (1H each, aromatic and olefinic protons), δ2.30 (3H, s, $-O-\overset{O}{\overset{\|}{C}}-CH_3$), δ1.70 (3H, s), δ1.42 (3H, s), δ1.10 (3H, s), and δ0.90 (3H, t, terminal methyl of side chain).

2. *Epoxidation of Δ^9-THC acetate.*—Small portions of *m*-chloroperbenzoic acid (200 mg) was added to 411 mg of Δ^9-THC acetate (**X**) in chloroform (10 ml) with stirring; the reaction mixture was left for 9 hours at room temperature. Excess reagent was destroyed with sodium sulfite solution. The reaction mixture was then transferred to a separatory funnel, and the organic layer was washed with 5% $NaHCO_3$, water and, finally, with saturated sodium chloride solution. The organic layer was dried over anhydrous magnesium sulfate and evaporated to dryness to give 9,10-epoxy-THC acetate as a red-orange oil (**XI**) (390 mg). Purity was established at >92.9% by glc on 2% OV-17 (RRT 0.48). It gave the following data: gc-ms (M^+ 372); ^{1}Hnmr ($CDCl_3$) δ6.45 and δ6.49 (1H each, s, aromatic protons), δ3.38 (1H, s, br), δ2.40 (3H, s, $-O-\overset{O}{\overset{\|}{C}}-CH_3$), δ1.43 (3H, s), δ1.35 (3H, s), δ1.06 (3H, s) and δ0.90 (3H, t, terminal methyl of side chain).

3. *Preparation of 9-OH-Hexahydrocannabinol* (**XII**).—To a 10 ml ethereal solution of 9, 10-epoxy-hexahydrocannabinol acetate (100 mg) was added 35 drops of 1M $LiAlH_4$ in ether, and the reaction mixture was stirred for 1 hour at room temperature. After work-up, a red oil (66 mg) was obtained. Purity was established at 89.7% by glc on 2% OV-17 (RRT 0.72). It gave gc-ms M^+ 332. The latter was purified on a preparative plate [0.5 mm thickness, solvent system: hexane-ethyl acetate (40:15)] to obtain 39.8 mg. It gave the following data: ms M^+ 332 (24%), 314 (68%), 299 (100%); ^{1}Hnmr ($CDCl_3$): δ6.26 (2H, s, aromatic protons), δ3.26 (1H, br, C_7-H), δ1.41 (3H, s), δ1.36 (3H, s), δ1.03 (3H, s) and δ0.90 (3H, t, terminal methyl).

B. SYNTHESIS OF THE 8-HYDROXY-ISO-HEXAHYDROCANNABINOL (8-OH-ISO-HHC)

1. *Preparation of Δ^8-iso-hexahydrocannabinol* (5).—To 639 mg of cannabidiol (**II**) in chloroform (35 ml) was added 15 ml of methanol containing 0.2 ml of concentrated sulfuric acid with stirring and cooling. The solution was left at room temperature for 3 days and was then worked up by the addition of water (200 ml); the resulting solution was extracted with ether (3 x 200 ml). The combined ethereal extract was washed with $NaHCO_3$ solution and, finally, dried over anyhydrous Na_2SO_4. Tlc of the latter, with hexane-ether (8:2) as a solvent system, showed the presence of 4 major spots (R_f values 0.09[3], 0.17[3], 0.39[3], and 0.52). Further fractionation of the above residue was carried out by column and preparative chromatography.

The compound with R_f value of 0.52 (compound **III**) was obtained as a yellow oil (50 mg) RRT was 0.35 on 2% OV-17. It gave the following data: gc-ms M^+ 314; ^{1}Hnmr ($CDCl_3$) δ6.16, δ6.01 (2H, s, aromatic protons), δ4.86 (2H, br, s, $C=CH_2$), δ3.33 (1H, m, C-3), δ2.33 (2H, t, benzylic protons of side chain), δ1.85 (3H, s, C-10 CH_3), δ1.25 (3H, s, C-1 CH_3) and δ0.88 (3H, s, terminal methyl of side chain). From ^{1}Hnmr data (6) compound A was identified as Δ^8-iso-hexahydrocannabinol (**III**).

2. *Conversion of Δ^8-iso-hexahydrocannabinol to its acetate.*—To 50 mg of (**III**) was added 0.2 ml each of pyridine and acetic anhydride, and the reaction mixture was left overnight. The reaction mixture, after work-up as previously described, yielded an oily material (com-

[3]Identified as the 2 isomeric 1-methoxy-hexahydrocannabinols, and 8-methoxyhexahydrocannabinol by ^{1}Hnmr and mass, respectively.

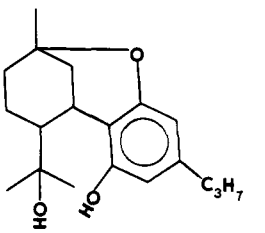
pound **VI**, 44 mg) shown to be one spot by tlc, R_f 0.58) using hexane-ether (8:2) as a solvent system. RRT was 0.35 on 2% OV-17 and 0.65 on 6% OV-1. It gave the following data: M^+ 356 (gc-ms). ^{1}Hnmr ($CDCl_3$) δ6.45, δ6.3 (1H each, s, aromatic protons), δ4.86 (2H, br, s, C=CH_2), δ3.4 (1H, m, C-3) δ2.5 (2H, t, benzylic protons) δ2.25 (3H, s, O-C-CH_3) δ1.81 (3H, s, C-10 CH_3), δ1.23 (3H, s, C-1, CH_3), and δ0.86 (3H, t, W-CH_3).

3. *Conversion of Δ^8-isohexahydrocannabinol acetate to 8-9-epoxy-isohexadydrocannabinol acetate* **VII**.—To a chloroform solution of Δ^8-isohexahydrocannabinol (44 mg) was added 20 mg of *m*-chloroperbenzoic acid portion-wise; the reaction was stirred for 48 hours at room temperature. Work-up, as mentioned previously, gave a yellow oil (**VII**) (wt. 38.5 mg) RRT was 0.57, 0.46 on 2% OV-17. The gc-ms gave for a molecular ion at M^+ *m/e* 372 (100%), 356 (36%), 330 (74%).

4. *Conversion of 8,9-epoxy-isohexahydrocannabinol acetate to 8-hydroxy-isohexahydrocannabinol* (**VIII**) (7).—To 16 mg of 8,9-epoxy-isohexahydrocannibol acetate in 2 ml of ether was added 1 ml of a 1.08 M $LiAlH_4$ solution. The reaction mixture was stirred for 1.5 hours and then decomposed by drop-wise addition of water and extracted with ether. The combined ethereal extracts (3 x 15 ml) were dried over anhydrous $MgSO_4$ and evaporated to dryness (wt 12 mg). Tlc of the residue on silica gel G with hexane-ether (8:2) as a solvent system showed a major spot (with R_f value 0.09). Preparative chromatography of the residue under the conditions mentioned above yielded **VIII** as a yellow oil (4 mg), RRT (2% OV-17 0.95). It gave the following data: ms, M^+ 332 (20%), 314 (22%), 299 (17%), 271 (23%), 258 (9%) 243 (3%), 231 (100%); ^{1}Hnmr ($CDCl_3$) δ6.11 and δ6.33 (1H, each, s, aromatic protons) δ3.30 (br, s, C-3 proton), δ2.3 (2H, t, benzylic protons of side chain) δ1.47 (6H, s, CH_3) δ1.26 (3H, s, CH_3) and δ0.88 (3H, t, ω-CH_3).

DISCUSSION

Cannabiglendol (**I**), a novel cannabinoid, was isolated from the leaves and small stems of an Indian variant of *Cannabis sativa* L. grown in Mississippi.



I

Compound **I** was obtained as a pale yellow oil after repeated chromatography. The ^{1}Hnmr showed signals for methyl groups at δ1.50 (6H, s) and δ1.30 (3H, s). In addition, the aromatic protons appeared at δ6.47 and δ6.38 (1H, each, s, br); the C-3[4] proton appeared at δ3.28 (1H, br); and the benzylic protons of the side chain appeared at δ2.54 (2H, t). These data indicated a cannabinoid having its methyl groups borne on oxygenated carbons. The mass spectrum showed a molecular ion at *m/e* 304 which loses a molecule of water to give an ion at *m/e* 286. Other characteristic ions were observed at 271, 243, and 203 (base peak). These mass spectral data indicated that the isolated compound was a hydroxy cannabinoid with a propyl side chain (as shown by base peak appearing at 203).

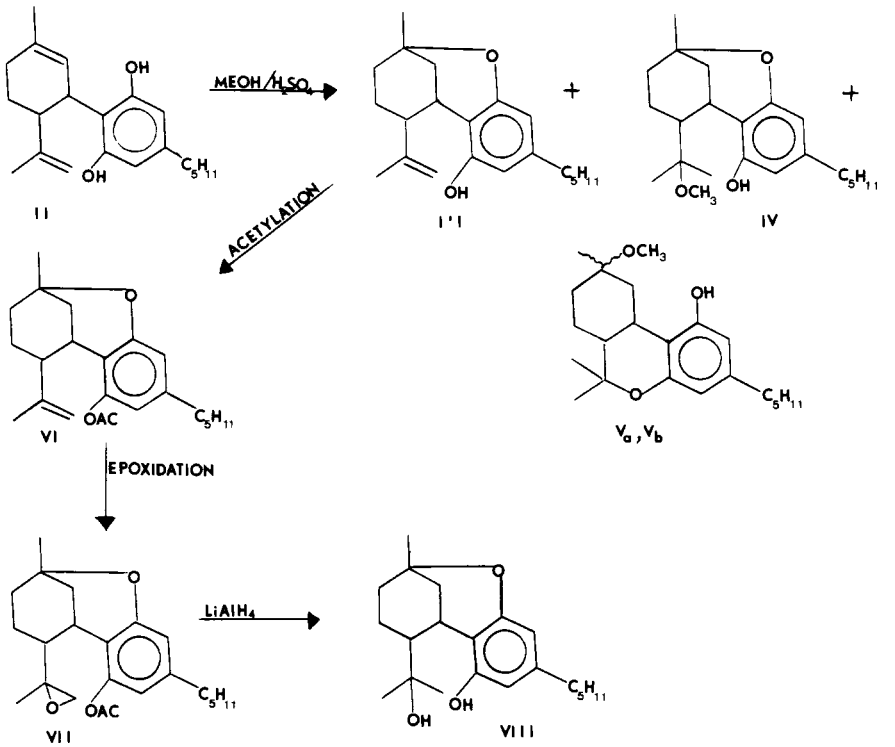
Since the starting chemicals are more readily available and since the spectral characteristics of the pentyl and propyl homologs would be similar enough for a comparative study, it was decided to synthesize the C_5-homolog **VIII** and its isomer **XII**.

To prepare the 8-hydroxy-isohexahydrocannabinol **VIII**, CBC **II** was reacted

[4]According to the terpene nomenclature.

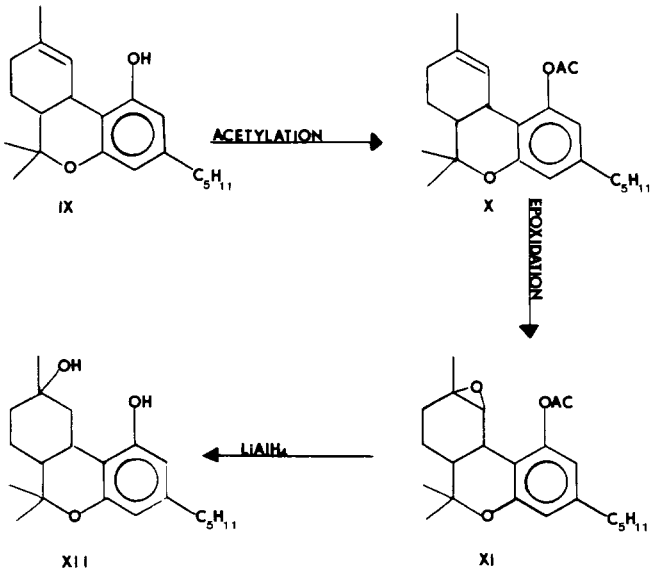
SYNTHESIS OF 8-HYDROXY-HEXAHYDROCANNABINOL

SCHEME 1



SYNTHESIS OF 9-HYDROXY-HEXAHYDROCANNABINOL

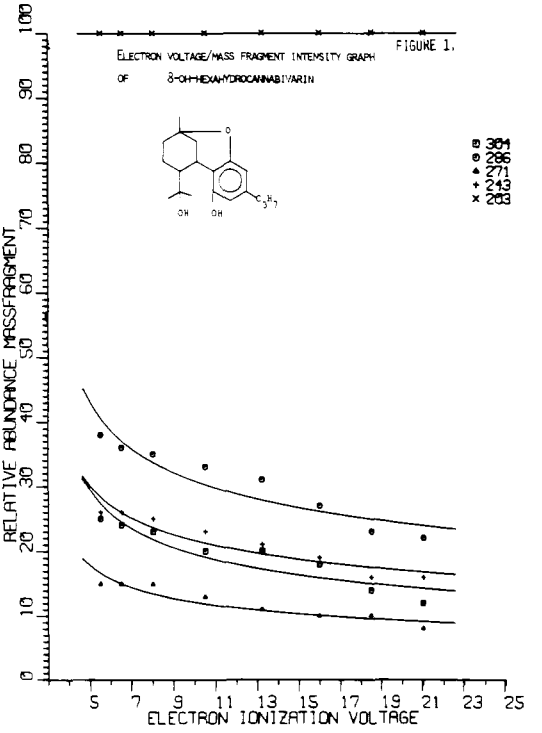
SCHEME 2

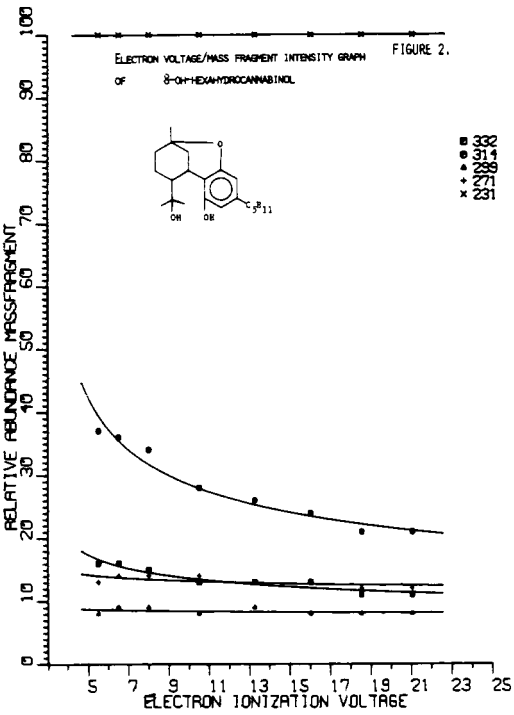


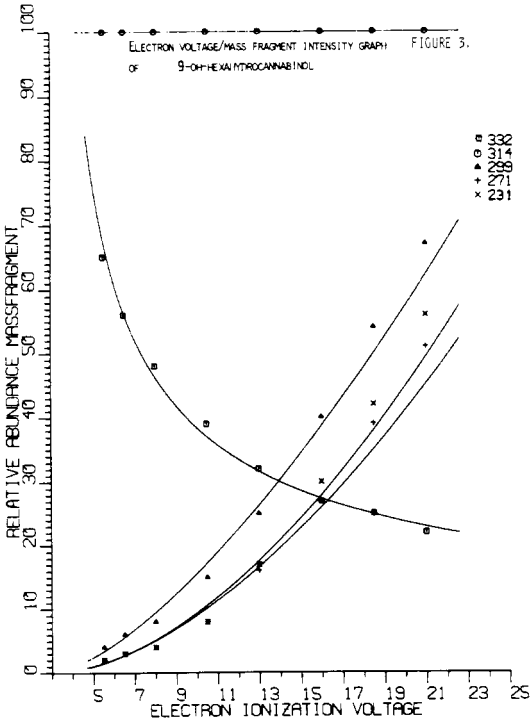
with MeOH/H_2SO_4 (scheme I) to yield four major components: **III**, **IV**, **Va** and **Vb**, which were identified (ms, ^{1}Hnmr) as Δ^8-isohexahydrocannabinol, 8-methoxy-isohexahydrocannabinol and the two isomeric 1-methoxy-hexahydrocannabinols, respectively. Trials for demethylating compound **IV** with different demethylating reagents, namely, BCl_3 (11), BBr_3 (11) and NaCN–DMSO (12) did not give the desired 8-hydroxy-isohexahydrocannabinol **VIII**; instead a mixture of **III** and the starting material **IV**, was obtained. So an alternative route was used (scheme I) which consisted of the acetylation of **III**, followed by epoxidation and then reduction with $LiAlH_4$ to give corresponding alcohol **VIII**. Similar to the C_3-natural material, the synthetic C_5-homolog was found to be much less stable than cannabinoids belonging to the dibenzopyran nucleus, i.e., Δ^9-THCV and Δ^9-THC. In the case of cannabiglendol (**I**), the decomposition products were relatively non-polar cannabinoids.

Compound **XII** was also synthesized in a way similar to that described in scheme II.

The ^{1}Hnmr spectrum of compound **XII** showed signals for a methyl group on oxygenated carbons at δ1.41 (3H, s), δ1.36 (3H, s) and δ1.03 (3H, s). On the other hand, the methyl signals in compound **VIII** appeared at δ1.47 (6H, s) and δ1.26 (3H, s). On comparing these data with that of **I**, δ1.50 (6H, s) and δ1.30 (3H, s), one could exclude structure **XII** on the basis of one of the methyl group being considerably shielded, and also from the mass spectrum which now shows a base peak of *m/e* 299. In addition, the mass spectral data of **VIII** showed an identical fragmentation pattern as in **I** with the molecular ion appearing at *m/e* 314, followed by other characteristic fragments at 299, 271, 243, and 231 (base




ELECTRON VOLTAGE/MASS FRAGMENT INTENSITY GRAPH
FIGURE 2.
OF 8-OH-HEXAHYDROCANNABINOL
332
314
299
271
231
RELATIVE ABUNDANCE MASSFRAGMENT
ELECTRON IONIZATION VOLTAGE


ELECTRON VOLTAGE/MASS FRAGMENT INTENSITY GRAPH
FIGURE 3.
OF 9-OH-HEXAHYDROCANNABINOL
332
314
299
271
231
RELATIVE ABUNDANCE MASSFRAGMENT
ELECTRON IONIZATION VOLTAGE

peak). Further proof that the structure in hand belongs to the iso-series with a propyl group in the side chain was obtained from the ev-mf graphs (8, 9, 10) of the natural and the two synthetic compounds **VIII** and **XII**. These graphs were obtained by recording the mass spectra at various ev-settings (5.5–21 ev) and then plotting the relative intensities of the characteristic ions against ev. See figures 1, 2, and 3. Comparison of the graphs showed that the graph of compound **VIII** has the same shape as that of **I**, with the exception that each mass fragment was 28 units less. As the main fragmentation takes place in the terpenoid part of the cannabinoid molecule, the conclusion must be that the isolated cannabinoid has a propyl side chain instead of the more prevalent pentyl side chain. Therefore compound **I**, the first naturally occurring C_3 isohexahydrocannabinol, was identified as 8-OH-isohexahydrocannabivarin[5] (cannabiglendol).

The synthetic C_5- homolog **VIII** had a relative retention time of 0.95 which is almost double the value of that of the C_3- natural homolog (0.49). These data are in agreement with the results obtained for Δ^9-THCV and Δ^9-THC, 0.26 and 0.49, respectively.

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[5]Chemical abstract name: 3,4,5,6,-tetrahydro-7-hydroxy-α,α-2-trimethyl-9-n-propyl-2, 6-methano-2H,1-benzoxocin-5-methanol. The stereochemistry of the major intermediates and product was not examined in this study.