is rationalized in this way, II-*endo*-3-*d* and *cis*-cyclopentyl-2-*d* brosylate should show larger isotope effects than II-*exo*-3-*d* and *trans*-cyclopentyl-2-*d* brosylate.

(15) National Science Foundation Cooperative Fellow, 1965–1969.

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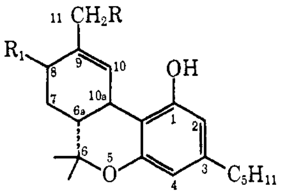
Isolation, Structure, and Biological Activity of Several Metabolites of Δ^9-Tetrahydrocannabinol

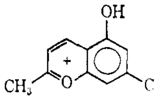
Sir:

Although there has been considerable recent interest in the metabolism of Δ^9-tetrahydrocannabinol,[1a,b] **1a**, none of the metabolites has been isolated or characterized. Since **1a** is the constituent largely responsible for the psychotomimetic properties of cannabis[2] (hashish, marihuana), the properties of its metabolites are of great importance in understanding the physiological disposition of this drug. We wish to report for the first time the *structure* and *biological activity* of several metabolites of **1a** produced by a rat liver microsomal fraction.

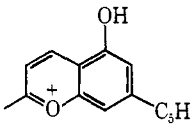


1a, R = R₁ = H
b, R = OH; R₁ = H
c, R = R₁ = OH
d, R = OAc; R₁ = H

Table I. Low Resolution Mass Spectra of Δ^9-THC and Metabolites

Assignment	Mass no. of ion from compd **1a**	**1b**	**1c**	**1d**
M	314	330	346	372
M − CH_3	299	315		
M − H_2O		312	328	
M − CH_2OH		299	315	
M − H_2O − CH_3		297	313	
M − CH_2OH − H_2O			297	
M − CH_3COOH				312
M − CH_3COOH − CH_3				297
M − C_3H_7	271			
M − H_2O − C_3H_7			285	
	231	231	231	231

Aerobic incubation of synthetic **1a**,[3] containing tritium-labeled **1a**[4] as a marker, with the 10,000*g* supernatant prepared from male rat liver homogenate to which was added appropriate cofactors,[5,6] followed by ethyl acetate extraction and chromatography on silica gel resulted in the isolation of unreacted **1a**, yield 25%,[7] and three new compounds, **1b**, mp 136.5–138°, yield 30%; **1c**, mp 139–140.5°, yield 30%; and **1d**, an oil, yield 15%. Tables I and II compare, respectively, the mass spectral and nmr data of **1a–d**. The structure of **1b**, the major metabolite, was obtained as follows: the uv spectrum [ν_{max}^{EtOH} 283 nm (1280), 276 nm (1250)] of **1b** was the same as that of **1a**,[2] indicating that the basic cannabinoid chromophore is retained in **1b**. High resolution mass spectrometry of **1b** gave a parent ion at *m*/*e* 330.2203, consistent only with the structure $C_{21}H_{30}O_3$ and showing that one hydroxyl moiety had been substituted for hydrogen in **1a**, $C_{21}H_{30}O_2$. A very strong base peak was found at *m*/*e* 299 (M − CH_2OH). In addition a very useful diagnostic peak at *m*/*e* 231 was noted. It has been reported[8] that this peak is due to the fragment



Its presence is reasonable evidence that the new hydroxyl group in **1b** cannot be located on the two rings shown, or on the amyl side chain. The strong base peak at *m*/*e* 299 is best accounted for by elimination of the C-9 vinylic hydroxymethyl group in **1b**. The structure assigned was completely confirmed by nmr analysis (Table II). Comparing **1a** and **1b**, it will be noted that the three-proton singlet at 1.62 ppm due to the C-9 methyl[2] of **1a** is *absent* in **1b** and replaced by a new two-proton signal at 3.92 ppm. The chemical shift of this signal is in good agreement for that expected for –C═C—CH_2OH. With these exceptions the nmr signals for **1a** and **1b** are virtually identical. The structure of the closely related compound **1d** was established along similar lines, the presence of the acetoxymethyl group at C-9 being readily established by mass spectral and nmr data, *cf.* Tables I and II, and ir analyses ($\nu_{max}^{CCl_4}$ 1740 cm^{-1}). Furthermore, careful alkaline hydrolysis converted **1d** to **1b**. Compound **1d**

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(1) (a) G. Joachimogliu, J. Kibaris, and C. Miras, *Propt. Acad. Athenon*, 70, 161 (1967); (b) S. Agurell, I. M. Nelsson, A. Ohlsson, and F. Sandberg, *Biochem. Pharmacol.*, 18, 1195 (1969).

(2) An excellent review of the literature on the chemistry and biological activities of the various cannabinoids is presented by R. Mechoulam and Y. Gaoni, *Fortschr. Chem. Org. Naturst.*, 25, 175 (1967).

(3) This material was obtained through Dr. John Scigliano, Center for Drug Abuse, NIMH, NIH, Bethesda, Md.

(4) M. L. Timmons, C. G. Pitt, and M. E. Wall, *Tetrahedron Lett.*, 3129 (1969).

(5) For recent reviews of microsomal hydroxylations and drug transformations, see: (a) "Microsomes and Drug Oxidations," J. R. Gillette, A. H. Conney, G. J. Cosmides, R. W. Estabrook, J. R. Fouts, and G. J. Mannering, Ed., Academic Press, New York, N. Y., 1969; (b) D. V. Parke, "The Biochemistry of Foreign Compounds," Pergamon Press, Elmsford, N. Y., 1968.

(6) Summary of experimental conditions: 500 g of liver was obtained from 350-g male rats, pretreated with phenobarbital prior to sacrifice. A homogenate was prepared using 0.1 *M* potassium phosphate buffer (5 l., pH 7.4), containing 0.013 *M* magnesium chloride. The ice-cold homogenate was centrifuged at 10,000 G. To the supernatant thus obtained was added 1.0 g of **1a** plus 394 μCi of tritium-labeled **1a** and cofactors, NADP (6.55 g, 1.6 × 10^{-3} *M*), G6P (14.3 g, 8.0 × 10^{-3} *M*), and G6P-dehydrogenase, 1000 units. The mixture was incubated aerobically, shaking for 2 hr at 37°, using 10 3-l. Fernbach flasks. The reaction was quenched by extraction with ethyl acetate.

(7) (a) The yields were based on thin layer chromatography of the ethyl acetate extract of the incubation mixture using a radioscanner to reveal the individual labeled peaks. Zones were scraped and counted in a liquid scintillation counter to determine the yields. (b) The same compounds, **1a–d**, were found, but with lower yields of **1b**, **1c**, and **1d**, in the case of rats which were not treated with phenobarbital.

(8) H. Budzikiewicz, R. T. Alpin, D. A. Lightner, C. Djerassi, R. Mechoulam, and Y. Gaoni, *Tetrahedron*, 21, 1881 (1965); U. Claussen, H. W. Fehlhaber, and F. Korte, *ibid.*, 22, 3535 (1966).

Table II. Proton Magnetic Resonance Spectra of Δ^9-THC and Metabolites[a]

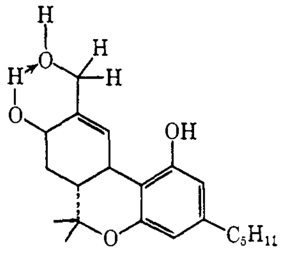
Assignment	Compd			
	1a	**1b**	**1c**	**1d**
C-10	6.50 (s)	6.72 (s)	6.81 (s)	6.83 (s)
C-2, C-4	6.23 (s)	6.24 (s)	6.25 (s)	6.22 (s)
	6.10 (s)	6.10 (s)	6.11 (s)	6.18 (s)
C-10a	3.20 (d, 10 Hz)	3.20 (d, 10 Hz)	3.30 (d, 10 Hz)	3.20 (d, 10 Hz)
Ph*CH*$_2$	2.40 (t, 7.5 Hz)	2.40 (t, 8 Hz)	2.42 (t, 7 Hz)	2.38 (t, 7.5 Hz)
C-9-Methyl	1.62 (s)			
C-9-Hydroxymethyl		3.92 (s)	4.12 (s)	
C-9-Acetoxymethyl				4.40 (s)
C-6-CH$_3$	1.35 (s)	1.36 (s)	1.38 (s)	1.34 (s)
	1.04 (s)	1.04 (s)	1.09 (s)	1.03 (s)
Side chain methyl	0.87 (t, 6 Hz)	0.87 (t, 6 Hz)	0.89 (t, 6 Hz)	0.85 (t, 6 Hz)
C-8			4.50 (t, 9 Hz)	

[a] All spectra were obtained in hexadeuterated acetone (because of the insolubility of **1b** in chloroform) using a Varian Model A100 nmr spectrometer. Results are given in parts per million; splitting and J values are in parentheses.

is most likely an artifact formed from **1b** during the extraction procedure.[9]

The structure of the most polar metabolite, **1c**, was assigned on the basis of the following considerations. As in the other compounds in this series, the basic cannabinoid uv spectrum was noted. The parent ion *m/e* 346.2228 agreed well with the formula $C_{21}H_{30}O_4$, consistent with substitution of two hydroxyl groups for hydrogen in **1a**. Two very intense daughter peaks at *m/e* 328 and 315 (M − 18 and M − 31, respectively) were noted, the former being the base peak. Another strong peak at *m/e* 297 was shown by metastable peak analysis to be derived from the *m/e* 315 peak by loss of water. The diagnostically useful peak at *m/e* 231, previously referred to, was again noted and meant that metabolic hydroxylation had occurred only in the cyclohexene ring. The mass spectral fragmentation pattern was thus in good agreement with the structural assignment in which both hydroxyl groups are allylic to the Δ^9-double bond. This assignment was confirmed by nmr analysis. As shown in Table II, the C-9 methyl signal of **1a** is absent in **1c** and replaced by a new peak at 4.12 ppm (2 protons). At the same time a new partially resolved triplet (1 H, ΣJ = 18 Hz) of diagnostic importance is found at 4.50 ppm. The remaining signals are identical with those found in **1a** and **1b**. The last named signal must be due to the HO–C–H moiety found only in **1c**. Molecular models of the structure assigned to **1c** indicate that *regardless of the configuration* and *conformation of the C-8 hydroxyl group*, there should exist *strong intramolecular hydrogen bonding* with the C-9 hydroxymethyl group. Infrared studies of **1c** using standard sequential dilution techniques showed unequivocally that the hydrogen bonding noted was indeed intramolecular.[10] The configuration and conformation of the C-8 hydroxyl group is under further investigation.[11]

Finally, preliminary behavioral and neuropharmacological screening indicates that **1b** is at least *equipotent* to **1a**, wheras **1c** is inactive.[12] The inactivation of **1c** may well be due to the previously noted intramolecular hydrogen bonding which could produce a structure, such as that shown below, incapable of fitting a required enzyme site. Further studies are underway at



this laboratory to ascertain whether similar metabolism occurs in other species, including man, under *in vitro* and in *in vivo* conditions.[13]

Acknowledgment. The studies reported were carried out with the support of Contract No. PH-43-69-62, National Institute of Mental Health, NIH, HEW. We wish to thank David Rosenthal and Fred Williams for the mass spectral data carried out at the Research Triangle Mass Spectroscopy Center under NIH Grant No. PO7 RR 00330-03 MCHA, Charles Fenske and Joseph

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(9) The ethyl acetate used contained a trace of acetic acid. During the evaporation of the large volume of solvent required for extraction we believe that partial acetylation of **1b** occurred. Supporting this observation is the fact that the liver microsomal fractions used by us contain active esterases. Thus in our laboratory similar rat liver preparations rapidly hydrolyze ethynodiol diacetate to ethynodiol.

(10) Compounds **1a** and **1b** both show in the ir-hydroxyl region only one band at 3590 cm^{-1}. In contrast, **1c** in CS_2 solution over a range of 0.1–2.0 mg/ml shows two band, one of relative weak intensity at 3410 cm^{-1} and the other of greater intensity at 3200–3250 cm^{-1}. The relative ratios were unchanged by dilution over a 20-fold range, demonstrating the existence of intramolecular bonding.

(11) It is tempting to assign an axial conformation to the C-8 proton on the basis of ΣJ = 18 Hz. However, lacking the other epimer, and considering the ring in question is cyclohexene rather than cyclohexane, we believe it is premature to make a rigid assignment of the stereochemistry.

(12) The procedures used to evaluate the activity of these compounds are based on the comprehensive procedure by S. Irwin, *Psychopharmacologia*, **13**, 222 (1968). In particular **1a** and **1b** showed similar losses of corneal reflex, decrease in spontaneous activity, and effective analgesic activity. Under these circumstances **1c** was completely inactive. All compounds were made up in 5% Tween-80 solution and were administered intravenously at a dose level of 10 mg/kg. A 5% Tween-80 solution was used as a solvent blank; in addition a "positive" blank containing 10 mg/kg of cannabidiol in 5% Tween-80 was administered. Each compound and the various blanks were administered to mice at the same time. The blanks were always negative.

(13) It is of interest that recent, parallel studies by Burstein, Mechouoam, *et al.* [*Nature*, **225**, 87 (1970); *J. Amer. Chem. Soc.*, **92**, 3468 (1970)] and Foltz, *et al.* (*Science*, in press) which deal with the metabolism of Δ^8-THC indicate that hydroxylation occurs at carbon-11 without shifting of the Δ^8 double bond and that the structure of the resultant compound is completely analogous to that of compound **1a**. Thus, hydroxylation of the C-11 double bond appears to be a major metabolic pathway in cannabinoid metabolism.

Younger for the nmr data, and Mrs. Joy Gidley for assistance in biological experiments.

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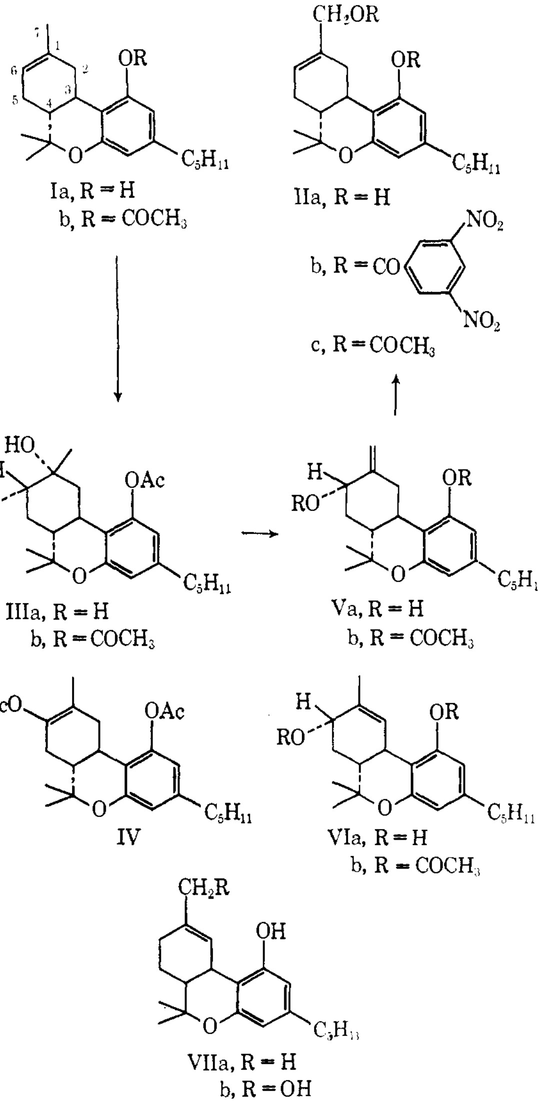
Identification through Synthesis of an Active $\Delta^{1(6)}$-Tetrahydrocannabinol Metabolite

Sir:

We recently reported[1] the isolation and partial elucidation of the structure of a $\Delta^{1(6)}$-tetrahydrocannabinol ($\Delta^{1(6)}$-THC) metabolite obtained from the urine of rabbits injected with $\Delta^{1(6)}$-THC (Ia) tritiated at C-2. On the basis of mass spectral analysis and certain chemical transformations, we deduced that the metabolite is a hydroxylated derivative of Ia. The new hydroxyl group was shown to be allylic, and we tentatively suggested structure IIa. The metabolite was only obtained in minute amounts which were not sufficient for a nuclear magnetic resonance determination; hence the problem was approached through synthesis. We report now that we have completed the preparation of IIa and have shown that it is identical with the metabolite.



Ia, R = H
b, R = COCH$_3$

IIa, R = H
b, R = CO
c, R = COCH$_3$

IIIa, R = H
b, R = COCH$_3$

Va, R = H
b, R = COCH$_3$

IV

VIa, R = H
b, R = COCH$_3$

VIIa, R = H
b, R = OH

Reaction of $\Delta^{1(6)}$-THC acetate (Ib)[2] with osmium tetroxide gave 1α,6α-dihydroxyhexahydrocannabinol acetate (IIIa) in 72% yield: mp 75–76°; $[\alpha]_D$ −121° (EtOH); δ (CCl$_4$) 1.10, 1.27, 1.38 (three methyl groups), 2.32 (acetoxyl methyl group), 3.50 (quartet, $J_{5\alpha,6\beta}$ = 11 Hz, $J_{5\beta,6\beta}$ = 4.5 Hz; C-6 proton), 6.35, 6.50 (two aromatic protons). On acetylation (acetic anhydride–pyridine) the diacetate IIIb is obtained (95%) as an oil, $[\alpha]_D$ −107° (EtOH). Dehydration of IIIb with thionyl chloride in pyridine yielded a mixture of three compounds: the enol acetate IV (31%; $[\alpha]_D$ −67° (EtOH); δ (CCl$_4$) 1.12, 1.36 (two methyl groups), 1.54 (one vinylic methyl group), 2.08, 2.21 (two acetoxyl methyl groups), 6.24, 6.41 (two aromatic protons), no peaks between 2.95 and 6.24; $\nu_{\max}^{\mathrm{CCl_4}}$ 1770 cm^{-1}) and the allylic acetates Vb and VIb. The last two compounds were not separated[3] but were converted with lithium aluminum hydride directly to a mixture which on chromatography gave pure Va (28% from IIIb; $[\alpha]_D$ −36° (EtOH); δ (CDCl$_3$) 1.07, 1.40 (two methyl groups), 3.87 (C-3 proton), 4.18 (br quartet, $J_{5\alpha,6\beta}$ = 11 Hz, $J_{5\beta,6\beta}$ = 6 Hz; C-6 proton), 4.97 (d, two C-7 protons), 6.07, 6.20 (two aromatic protons); $\nu_{\max}^{\mathrm{CCl_4}}$ 909 cm^{-1} (terminal methylene group)) and VIa (21%; $[\alpha]_D$ −115° (EtOH); δ (CDCl$_3$) 1.10 and 1.40 (two methyl groups), 1.81 (vinylic methyl group), 4.15–4.60 (br, C-6 proton), 6.20, 6.28 (two aromatic protons), and 6.60 (C-2 vinylic proton)).

Treatment of Va with boron trifluoride etherate in methylene chloride caused an allylic rearrangement forming IIa (10%; $[\alpha]_D$ −255° (EtOH); δ (CDCl$_3$) 0.98, 1.35 (two methyl groups), 4.06 (br s, two C-7 protons), 5.7 (C-6 proton), 6.08, 6.21 (two aromatic protons)), 7-OH-$\Delta^{1(6)}$-THC bis-3,5-dinitrobenzoate (IIb) (mp 140–142°; δ (CDCl$_3$) 1.20, 1.40 (two methyl groups), 4.65 (two C-7 protons), 5.88 (one olefinic proton), 6.53, 6.65 (two aromatic protons)), 7-OH-$\Delta^{1(6)}$-THC diacetate (IIc) (δ (CDCl$_3$) 1.12, 1.38 (two methyl groups), 2.07, 2.30 (two acetoxyl methyl groups), 4.48 (two C-7 protons), 5.77 (one olefinic proton), 6.42, 6.57 (two aromatic protons)).

Direct comparisons of IIc with the acetylated tritiated metabolite[4] were made by thin layer (tlc) and gas chromatography (glpc) and by mass spectral analysis under identical conditions. More than 90% of the radioactivity was found to migrate with the synthetic material (IIc) on tlc using 80% hexane–20% acetone as the eluent; similar results were obtained when a second chromatogram using 48% benzene–48% hexane–4% methanol was run. Identical retention times were observed on glpc (separately and as a mixture)

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[1] S. H. Burstein, F. Menezes, E. Williamson, and R. Mechoulam, *Nature*, **225**, 87 (1970).

[2] Review: R. Mechoulam and Y. Gaoni, *Fortschr. Chem. Org. Naturst.*, **25**, 175 (1967).

[3] When separation was attempted Vb could be obtained in low yield: mp 88–89°; $[\alpha]_D$ −66° (EtOH); δ (CCl$_4$) 1.06, 1.36 (two methyl groups), 2.07, 2.25 (two acetoxyl methyl groups), 4.77 (two terminal methylene protons, br s), 5.15 (C-6 proton, quartet, $J_{5\alpha,6\beta}$ = 11 Hz, $J_{5\beta,6\beta}$ = 6 Hz), 6.25, 6.40 (two aromatic protons); $\nu_{\max}^{\mathrm{CCl_4}}$ 908 cm^{-1} (terminal methylene group).

[4] The material used for these comparisons was obtained by incubation of Ia with a homogenate of rabbit liver; the details will be reported later in a full paper. The *in vivo* and *in vitro* metabolites were shown to be chromatographically identical.