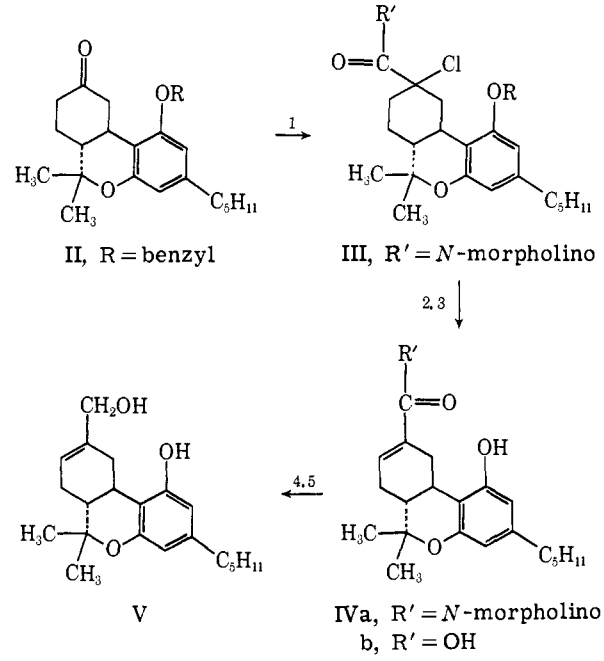
The synthesis involves allylic halogenation of Δ^9-THC acetate with sulfuryl chloride, followed by acetoxylation with silver acetate. Thus, redistilled sulfuryl chloride (1 mol equiv) was added to a stirred solution of Δ^9-THC acetate (8.7 g) in carbon tetrachloride (80 ml) at room temperature over a period of 5 min. After approximately 60 min (glc showed quantitative reaction), the solvent was evaporated *in vacuo* and replaced by 1.4 *M* silver acetate in acetic acid (1.5 mol equiv). After 24 hr, the mixture of crude acetoxylated products was isolated and saponified with aqueous ethanolic potassium hydroxide at room temperature (16 hr). Gradient elution from silica gel with an acetone–benzene mixture first afforded 8β-hydroxy-Δ^9-THC (1.2 g, 14%), followed by 8α-hydroxy-Δ^9-THC (80 mg, 1%), and finally 11-hydroxy-Δ^9-THC (415 mg, 5%). The 8β- and 11-hydroxylated products were spectroscopically identical with authentic samples.[1a-b,5] 8α-Hydroxy-Δ^9-THC was identified on the basis of its high-resolution mass spectrum [*m/e* 330.219 (calcd for $C_{21}H_{30}O_3$, 330.219), 315 (M − CH_3), 312 (M − H_2O), 311 (M − H_2O–H), 297 (M − H_2O–CH_3), 231 (M − H_2O–C_6H_9)] and comparison of the nmr spectrum with published data.[9]

A number of other allylic halogenating and oxygenating reagents[10] have been examined, with the object of exploiting any variation in selectivity of attack at the primary and secondary allylic sites of Δ^9-THC. *N*-Bromo- and *N*-chlorosuccinimide, triethylcarbinyl hypochlorite, and molecular chlorine all produce predominantly 8β-hydroxy-Δ^9-THC after acetoxylation and saponification, with only minor amounts of 11-hydroxy-Δ^9-THC. Palladium acetate[11] and *tert*-butyl peracetate afford only cannabinol. The use of 2 equiv of sulfuryl chloride leads to reduced yields of the monohydroxy metabolites and no 8,11-dihydroxy-Δ^9-THC, apparently because of competing aromatic chlorination after the introduction of the first chlorine atom. The very efficient[1] conversion of Δ^9-THC to 11-hydroxy-Δ^9-THC *via in vitro* microsomal enzymatic hydroxylation emphasizes the uniqueness of the biochemical mechanism.

An alternate, more lengthy, synthesis of 11-hydroxy-Δ^9-THC failed because of unexpected loss of stereochemical control, but instead provided a new[12] route to 11-hydroxy-Δ^8-THC (V, Scheme I), a physiologically active metabolite of Δ^8-THC. Thus, preparation of the α-chloramide III from the reaction of the morpholino enamine of 11-nor-9-ketohexahydrocannabinol *O*-benzyl ether[13] (II) with trichloroacetic acid,[14] followed by cleavage of the benzyl protecting group and conversion to the phenoxide anion to effect elimination of HCl, gave only the Δ^8-amide (IVa). Despite precedent,[15] no Δ^9-amide, which would be derived from intramolecularly assisted elimination, was formed. Saponification of IVa afforded the carboxylic acid IVb which, when reduced with lithium aluminum hydride, gave 11-hydroxy-Δ^8-THC in *ca.* 33% overall yield. This synthetic approach is likely to be of value because of the belief[16] that carboxylic acids related to IVb are involved in the general metabolic degradation of tetrahydrocannabinols.

Scheme I. Conversion of 11-Nor-9-ketohexahydrocannabinol to 11-Hydroxy-Δ^8-THC[a]



[a] Reagents: 1, morpholine–trichloroacetic acid; 2, HCl–$ZnCl_2$·$2H_2O$–$CHCl_3$; 3, Et_3COK–toluene, 0°; 4, aqueous EtOH–KOH; 5, $LiAlH_4$.

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the difficulty of removing all toxic selenium makes this impractical for pharmacological purposes. Other less direct, individual syntheses of 8α- and 8β-hydroxy-Δ^9-THC have recently been reported.[6,9]

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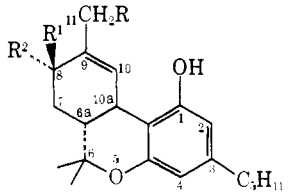
Identification of Δ^9-Tetrahydrocannabinol and and Metabolites in Man

Sir:

In recent years there has been unprecedented interest in the chemistry, metabolism, and pharmacology of

Δ^9-tetrahydrocannabinol (**1a**, Δ^9-THC), the agent be-



1a, $R = R_1 = R_2 = H$
b, $R = OH$; $R_1 = R_2 = H$
c, $R = R_2 = OH$; $R_1 = H$
d, $R = R_2 = H$; $R_1 = OH$
e, $R = R_1 = H$; $R_2 = OH$

lieved to be responsible for the psychotomimetic action of marihuana and hashish.[1a-d] A number of studies on the metabolism of Δ^8- and Δ^9-THC have been conducted *in vitro* and the structures of some pure metabolites determined by reliable procedures such as mass spectrometry and nmr.[2a-g] In contrast, *in vivo* metabolic studies with animals[3a-c] and more recently with man[4a-c] have employed less reliable techniques.[5] This communication presents for the first time rigid analytical identification of **1a**, 11-hydroxy-Δ^9-THC (**1b**), and 8α,11-dihydroxy-Δ^9-THC (**1c**), in the blood plasma of human volunteers.[6a,b] Furthermore, because of the availability of appropriate standards,[7] *8β- and 8α-hydroxy-Δ^9-THC* (**1d** and **1e**, respectively) have been tentatively identified for the *first time in man.* These metabolites have not been previously found *in vivo* in any species.

A total of 1200 ml of plasma from five volunteers obtained 6 hr after oral administration of **1a** was extracted with diethyl ether[8] to give 4.5 g of solids with an estimated cannabinoid concentration of 0.003% (by radioactivity determination). Subsequent purification by methanol precipitation of noncannabinoids, followed by chromatography on Sephadex LH-20, raised the concentration to 0.65%. This mixture of Δ^9-THC and metabolites was further purified by gradient elution from a precalibrated column of thin-layer grade silica gel using pressure column chromatography.[9] Material obtained from the column with an elution time corresponding to **1a** was subjected to gas–liquid chromatography–mass spectrometry.[10] A major component was identified as Δ^9-THC by comparison of its retention time and mass spectral fragmentation pattern with that of an authentic sample. A cannabinoid with the same molecular weight (*m/e* 314) but different retention time (6.5 *vs.* 13 min for Δ^9-THC) was also noted in the same sample. The ratio (1:1) of the intensities of the M − 15 fragment at *m/e* 299 and the molecular ion at *m/e* 314[11] together with the glc retention time of this component rule out the possibility that it is Δ^8-THC. The absence of a strong fragment at *m/e* 231 suggests structures related to $\Delta^{6a,(10a)}$-THC, Δ^{10}-THC, etc.[11] The key point is that sizable quantities of this THC isomer are present in plasma and would not be *noted* by less rigid methods, for example, employing only thin layer chromatography. In similar fashion the 11-hydroxy and 8α,11-dihydroxy metabolites, **1b** and **1c**, respectively, were positively identified as their trimethylsilyl derivatives by glc–mass spectrometry and comparison with authentic specimens. Using thin layer chromatography[12] and comparisons of radiolabeled material from plasma with authentic reference materials available through microsomal hydroxylation[2b] or synthetic techniques,[7] two new THC metabolites were noted for the first time in man and are tentatively identified as 8β- and 8α-hydroxy-Δ^9-THC (**1d** and **1e**, respectively).[13]

The role of 8-hydroxylated Δ^9-THC metabolites has not received sufficient recognition. Wall[2e] originally described the isolation and structure of the 8β metabolite **1d** after *in vitro* rabbit liver metabolism. The biological activity of synthetic **1d** in the monkey has recently been reported[14] and speculations on the possibility of its occurrence in man were presented. Recent

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(5) The methodology used in *in vivo* metabolism of Δ^9-THC cited in ref 3a–c and 4a–c has utilized thin layer chromatographic techniques on crude solvent extracts of plasma, urine, or feces. In some cases only only Δ^9- and 11-hydroxy-Δ^9-THC were available as comparison standards; in others the metabolites were not identified.

(6) (a) Protocols for the administration of radiolabeled Δ^9-THC orally to volunteers were approved by a joint NIMH–FDA Committee and by committees of the School of Medicine, University of North Carolina. (b) Subjects received a total of 35 mg of **1a** in sesame oil in capsules orally. Tritium-labeled **1a** [M. L. Timmons, C. G. Pitt, and M. E. Wall, *Tetrahedron Lett.*, 3129 (1969)], 50–100 μCi, was premixed with unlabeled **1a**. Samples of blood, urine, and feces were obtained at periodic intervals. The subjects were under medical supervision for at least 24 hr after receiving oral dosage.

(7) C. G. Pitt, F. Hauser, R. L. Hawks, S. Sathe, and M. E. Wall, *J. Amer. Chem. Soc.*, 94, 8578 (1972).

(8) The ether extract contained 50–60% of the total cannabinoids present—the remainder being conjugated. The conjugated fraction is under investigation.

(9) Conditions: 67 g of HF silica gel (Brinkmann), 60 lb of pressure in a 1-in. diameter glass column (Chromatronix pump and column system), gradient cyclohexane–chloroform (1:1) to chloroform to acetone. The column was precalibrated with known standards and chromatographic fractions taken corresponding to the volumes at which various standards were eluted.

(10) LBK-9000 glc–mass spectrometer with mass marker, 1% OV17 column, 210°, 35 ml of N_2/min.

(11) V. Claussen, H. W. Fehlhaber, and F. Korte, *Tetrahedron*, 22, 3535 (1966).

(12) The tlc chromatographic conditions are as follows: solvent system, acetone–chloroform (2:8); plates, E. Merck prepared silica gel on glass, 0.2 mm thick; development time, 2 hr; R_f: 1a, 0.92; **1d**, 0.72; **1e**, 0.55; **1b**, 0.46; **1c**, 0.10.

(13) From the R_f values given in ref 12, it can be seen that all the metabolites 1a–1e can be easily distinguished when a mixture of pure components is subjected to thin layer chromatography. The R_f differences between the epimeric 8α and 8β metabolites, **1e** and **1d**, respectively, is surprising. In practice, using crude plasma preparations there is slight overlap between the 11-hydroxy metabolite **1b** and the 8α-hydroxy metabolite **1e**, although with care the two compounds are separable. The relative R_f values of the monohydroxylated metabolites identified in plasma as **1b**, **1d**, and **1e** (and confirmed in the case of **1b** by glc–mass spectrometry) are quite similar to the values found in the pure mixture.

(14) Z. Ben-Zvi, R. Mechoulam, H. Edery, and G. Porath, *Science*, 174, 951 (1971).

studies in this laboratory[15] indicate that **1d** has one-fourth to one-half the potency of **1a** in mice, but with a much shorter duration of action, whereas the 8α metabolite **1e** is much less active. These findings in man confirm our previous postulates, based on *in vitro* studies,[2e,f] that Δ^9-THC is primarily metabolized by allylic hydroxylation at either the 8 or 11 position, followed by dihydroxylation to produce the 8,11-dihydroxy metabolite, and lends weight to the interesting speculations of Ben-Zvi, *et al.*,[14] that the cumulative effect of smoking marihuana may be based on a number of psychologically active cannabinoids derived from microsomal hydroxylation of Δ^9-THC. The number of these metabolites found in biological fluids or tissues after *in vivo* metabolism in animals or man, often with similar R_f values by thin layer chromatography, indicates that identifications made by this useful technique must be regarded as tentative until confirmed by more rigid techniques such as gas–liquid chromatography combined with mass spectrometry.

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(15) These studies were carried out by Dr. H. D. Christensen, Jr., whom we thank for giving us his data prior to publication elsewhere.

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Deuterium Labeling as a Probe for Signal Assignments and Mechanistic Studies by ^{13}C Nuclear Magnetic Resonance. Cationic Rearrangements in Polycyclic Systems[1]

Sir:

Initially, D-labeled compounds were utilized in ^{13}C studies as assignment aids[2] or to simplify the analysis of complex spin systems[3] since the operating conditions led either to the "disappearance" of the signal(s) for the deuterated carbon or restricted the observable effects to the absorption of that carbon. With Fourier-transform operation and proton noise-decoupling, however, the effects of a single deuterium atom are readily detected at neighboring carbons because of coupling and geminal isotope shifts.[4] In general,[5] $J_{CCCH} > J_{CCH}$ and, since $J_{CD} = J_{CH}$ (6.5), both vicinal and geminal ^{13}C–^2H couplings are observable; also in favorable cases vicinal coupling is identifiable because of the dihedral angle dependence. Thus, several features in ^{13}C spectra of ^2H-labeled species are monitors of the label and are readily utilized for structural and mechanistic purposes as illustrated below.

The spectrum of camphor-*3-exo-d*$_1$ exhibits a triplet for C-5 by vicinal coupling between C-5 and 3-exo-D, as well as the expected changes in the C-3 absorption, whereas C-7, also vicinal, is little affected; upon introduction of a 3-endo deuterium, the C-7 signal becomes a triplet. Thus, the vicinal interactions display the expected stereospecificity. The corresponding ^{13}C–^1H couplings for C-7 in hexachloronorbornenes are 9 and 0 Hz.[6] Carbons geminal to deuterium exhibit isotope shifts of 0.12 ± 0.04 ppm that are upfield for sp^3 carbons but downfield for carbonyl carbon.[7]

The assignments for the four 5,6-dimethylnorbornan-2-ones are straightforward with the exception of the C-4, C-5, and C-6 signals. This problem is resolved, however, by base-catalyzed D exchange of the C-3 proton(s). The spectra of the monodeuterated ketones reveal a 0.1-ppm upfield shift for one of these signals and a significantly broadened band for another, while the third is unaffected; thus the assignments to C-4, C-5, and C-6, respectively, were completed.

The careful selection of operating parameters leads to total integrated intensities of partially deuterated methyl and/or methylene carbons equal to those for nonlabeled materials since the Overhauser enhancement from proton decoupling is independent of the number of hydrogens.[8] The decrease in intensity for the *residual* CH absorption of a partially deuterated methine carbon gives a direct measure of the extent of deuteration. The ^{13}C–^2H induced triplets do not overlap the residual ^{13}C–^1H absorptions because of the isotope shift and coupling. If dipole–dipole relaxation is dominant for several carbons their integrated intensities are equal. For such systems the ^2H content at individual carbons can be assayed. These may be summed and compared with mass spectrometric data for total ^2H content as a check, and, in certain cases, proton spectra provide an independent measure for specific centers. Thus, with both quantitative and qualitative assay of the ^2H label(s), ^{13}C nmr is a new mechanistic probe.

Rearrangements of the norbornyl skeleton during acetolysis have been investigated with the aid of ^{14}C and ^3H labeling, and the tracer distribution determined by stepwise degradation of the product.[9,10] Similar information is obtained much more readily by direct observations with ^{13}C nmr. Brosylates **1** and **2**,

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