complementary structure and the semiconservative replication of DNA. The occurrence of exclusively complete mutants in *Ulva* may therefore be due to processes which transform or interfere with the primary alterations in DNA.

For procaryotic cells and fungi several hypotheses have been advanced to explain the formation of a complete mutant from a primary mutational event which affects only one of the DNA strands (*7*). The occurrence of exclusively complete mutants can be accounted for by the "master-strand hypothesis" (*1*) and the "repair hypothesis" (*8*). However, the former seems to ignore the semi-conservative replication of DNA, by assuming that only one of the DNA strands—the "master-strand"—serves as a template during replication. The "repair hypothesis" postulates that the primary mutagenic event results in mismatched bases in the DNA double helix and that a repair mechanism is engaged in replacing mismatched bases with matching ones. A primary mosaic mutation may thereby be transformed either back to the wild-type condition or to a complete mutation. According to this hypothesis absence of mosaic mutants should then indicate a high efficiency of the repair mechanism.

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3. One thousand milliliters of sea water, 50 ml of soil extract, 0.1 g of $NaNO_3$, and 0.02 g of $Na_2HPO_4 \cdot 12H_2O$.
4. According to the manufacturer, 90 percent of the output is at 253.7 nm.
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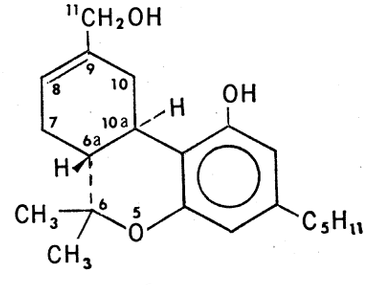
Metabolite of (−)-trans-Δ^8-Tetrahydrocannabinol: Identification and Synthesis

Abstract. *The major metabolite of* (−)-trans-Δ^8-*tetrahydrocannabinol observed in vivo and formed by hepatic microsomes in vitro is 11-hydroxy*-trans-Δ^8-*tetrahydrocannabinol. The metabolite was identified spectroscopically and was synthesized from* trans-Δ^8-*tetrahydrocannabinol. In tests with rats, the metabolite produced behavioral effects similar to those imparted by* Δ^8- *and* Δ^9-*tetrahydrocannabinol.*

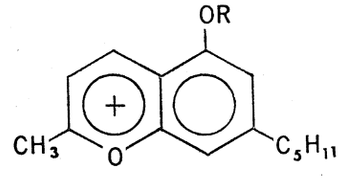
Increasing use of marijuana has generated interest in the metabolic fate of its psychotomimetically active constituents, (−)-*trans*-Δ^8-tetrahydrocannabinol (Δ^8-THC) and (−)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC) (*1*). While studying the distribution in the organs of rats after injection of ^{3}H-labeled Δ^8-THC, we observed several metabolites in the liver homogenates by means of thin-layer chromatography (TLC) (*2*). In subsequent studies, with ^{14}C-labeled Δ^8-THC, approximately 13 percent of the radioactivity was found in the liver 30 minutes after injection. Of the labeled material in the liver, approximately 65 percent corresponded to the major metabolite. The major metabolite of Δ^8-THC produced in vivo has the same R_F value in several TLC systems as the metabolite formed in vitro in fortified rat liver microsomes. The major metabolite produced in vitro has been identified as compound **1** and has been synthesized from Δ^8-THC.



1

The metabolism of Δ^8-THC in microsomes from rat liver was investigated by the procedure of Dixon *et al.* (*3*). After incubation of the microsomal system containing Δ^8-THC, the major portion of the metabolites was isolated by extraction with ethyl acetate. The major metabolite was separated by TLC on alumina, with 3 percent methanol in chloroform as solvent ($R_F = 0.3$; visualization reagent, fast blue B solution).

High-resolution mass spectra of the metabolite [$M^+ = C_{21}H_{30}O_3$; mass to charge (m/e), 330] and its bis-trimethylsilyl (TMS) derivative ($M^+ = C_{27}H_{46}O_3Si_2$; m/e, 474) indicated that it corresponds to monohydroxylated Δ^8-THC. Both mass spectra show prominent peaks corresponding to ion structure **2**, which results from a retro-Diels-Alder fragmentation of the molecular ion (*4*). Therefore, the hydroxylation must have occurred on that portion of the molecule lost during formation of fragment ion **2**.



2

Fragment ion from **1**: R=H, m/e 231
Fragment ion from TMS-derivative of **1**: R=Si(CH$_3$)$_3$, m/e 303

Comparison of the nuclear magnetic resonance (NMR) spectrum of compound **1** with the spectra of related compounds provided evidence sufficient for a complete structural assignment (*5*). In the NMR spectrum of Δ^8-THC, the protons of the methyl attached at C-9 show a resonance at 1.68 ppm. This resonance is absent in the spectrum of compound **1**. Instead, there is a new resonance at 4.04 ppm having a relative area corresponding to two protons. The chemical shift of these protons is consistent with the shift expected of a β-unsaturated primary alcohol. The diacetate of compound **1** was prepared with acetic anhydride in pyridine and purified by column and thin-layer chromatography. The NMR spectrum of the diacetate is very similar to that of compound **1** except for the acetate methyl resonances, which appear at 2.04 and 2.26 ppm, and a two-proton resonance, which now appears at 4.50 ppm. We attribute the two-proton resonance to the protons on the carbon bearing the acetoxy group, which conforms with a shift of 0.46 ppm downfield relative to the resonance of protons on the carbon bearing the hydroxyl

Table 1. Vinyl proton chemical shifts (ppm).

Parent compound	Acetate derivative	Spectral shift
Δ^9-THC, 6.38	6.01	+0.37
Δ^8-THC, 5.44	5.44	0.00
1, 5.72	5.80	−0.08

group in compound **1**. This acylation shift is characteristic of a primary alcohol (*6*). The integral lends further support to this assignment. We therefore conclude that the additional hydroxyl group is attached at C-11 with β-unsaturation.

The only remaining question regarding the structure of the metabolite was whether the olefinic bond has remained in the Δ^8 position or migrated to the Δ^9 position. A comparison of the NMR spectra of Δ^8-THC, Δ^9-THC, the metabolite, and their acetate derivatives showed that the double bond had not migrated (Table 1).

The large downfield shift of the vinyl proton in Δ^9-THC is attributed to deshielding caused by the anisotropy of the aromatic ring (*7*). Formation of the acetate changes the anisotropy of the aromatic ring, and the position of the vinyl proton resonance shifts upfield 0.37 ppm (*8*). In Δ^8-THC these effects are absent because of the remoteness of the vinyl proton from the aromatic ring. The resonance of the vinyl proton of the metabolite (**1**) is only slightly downfield from that of the vinyl proton of Δ^8-THC, a shift consistent with substitution at the allylic carbon. In addition, the position of the resonance of the vinyl proton in the diacetate of compound **1** has shifted only slightly relative to the resonance of the corresponding proton in compound **1**. Furthermore, the shift is opposite in nature to that observed in the Δ^9-THC system. We conclude that the vinyl proton is not at C-10 and therefore must be at C-8.

Synthesis of compound **1** from Δ^8-THC was achieved by a two-step conversion. The *trans*-Δ^8-THC was oxidized with selenium dioxide in 95 percent aqueous ethanol. Efforts to purify and characterize the major component of the oxidation were unsuccessful. Therefore, the crude product from the reaction was reduced directly with sodium borohydride in ethanol. The retention time by gas-liquid chromatography of the trimethylsilyl derivative of the resulting major product is identical to that of the TMS-derivative of the metabolite (**1**). The major product was separated by column chromatography on alumina and further purified by preparative TLC. The R_F values of the synthetic and natural metabolite are identical in several TLC systems. The mass spectra of the natural metabolite and the synthetic product are identical, as are the mass spectra of the corresponding trimethylsilyl derivatives. The NMR and infrared spectra of the natural and synthetic materials are also essentially identical.

Psychopharmacological tests indicate that the metabolite (**1**) exhibits a behavioral profile in the rat very similar to that exhibited by the Δ^8- and Δ^9-tetrahydrocannabinols with the use of the tests described by Irwin (*9*).

Burstein, Mechoulam, and co-workers have recently reported the isolation of a similar, if not identical, metabolite from rabbits injected with Δ^8-THC (*10*). The diacetate derivative of compound **1** gives a mass spectrum which is consistent with the mass spectral data for the diacetate derivative of the rabbit metabolite. These authors suggested that the additional hydroxyl group is located at the C-11 position (*10*). However, they could not exclude the possibility of the hydroxyl being located at the C-7 position.

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11. Supported by NIMH contract PH-43-68-1338. We thank Drs. R. Adams, H. M. Grotta, L. A. Mitscher, and C. W. Waller for helpful discussions.

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On the Apparent Homology of Actin and Tubulin

Abstract. *Muscle actin and ciliary A-tubulin from* Pecten irradians *have molecular weights of 46,000 and 59,000, respectively, as measured by sodium lauryl sulfate–polyacrylamide-gel electrophoresis. Tryptic and chymotryptic peptides obtained from these proteins are very dissimilar, indicating that there is little significant homology.*

Muscle actin and the microtubule structural protein tubulin (*1*) are similar in amino acid composition and in the presence of a bound nucleotide (*2–4*). These factors, coupled with the fact that both proteins apparently interact in some manner with adenosine triphosphatase proteins of motile systems, suggest that there may be some common structural basis for these similarities.

At one time, actin and tubulin were each thought to have a molecular weight of 60,000, but data based on the 3-methylhistidine content of actin has shown that the molecular weight of this protein is about 48,000 (*5*). Tubulin is devoid of 3-methylhistidine, and molecular-weight determinations by hydrodynamic methods have consistently given values near 60,000 (*2*, *4*).

Although early reports indicated a total of 1 mole of bound guanosine diphosphate (GDP) or guanosine triphosphate (GTP) per 60,000 g of tubulin (*3*), it now appears that 1 mole of nucleotide per 120,000 g is tightly bound while a second mole is apparently exchangeable with the medium (*6*). Actin was originally reported to contain 1 mole of bound adenosine diphosphate (ADP) per 57,000 g of protein, but more recent data show 1 mole per 45,000 to 47,000 g (*7*), basically in accord with the molecular-weight data discussed above.

With respect to amino acid composition, it has been pointed out that *Pecten* actin and *Tetrahymena* tubulin were no more dissimilar than actins derived from different species (*2*). However, when the apparent homology was further investigated in the same species, it was found that tubulin from the outer fibers of gill cilia of the scallop *Pecten* resembled actin from the striated adductor muscle far less than did these proteins resemble their true homologs from unrelated species (*8*). Results of fractionation of outer fiber doublets into the closely related subfiber com-