the valinomycin-induced K^+ conductance in this model system. Is this phenomenon related to the effect of DDT on K^+ conductance in axons? The apparent parallel is attractive but will not be fully established until it can be shown that analogs of DDT which are inactive on axons are inactive on membranes treated with valinomycin. One would like also to reproduce the DDT effect on an experimental membrane derived from axonic material.

Dieldrin, lindane, and DDT are potent against an adenosine triphosphatase of nervous tissue commonly held to be associated with, and perhaps identical to, the energy-requiring "sodium-potassium pump" (*10*). Such an action is entirely distinct from the effects on ion "'gates." It appears unlikely that an effect on the pump could be the cause of neurotoxic actions of these compounds, because such an effect would cause depression and, ultimately, failure of axonic transmission [as occurs in poisoning of nerve by azide, dinitrophenol, or cyanide (*11*)], rather than the excitatory effect which is observed experimentally.

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Mosaic Mutants: Absence in a Eucaryotic Organism

Abstract. *Exposure of procaryotic and eucaryotic cells to mutagenic agents generally gives both complete mutants and mosaic mutants. Irradiation of the eucaryotic multicellular alga* Ulva mutabilis *with ultraviolet light has given exclusively complete mutants.*

A cell in which a primary mutational event has taken place may give rise either to mutant cells only, or to both mutant and wild-type cells. In the former case, the mutation is said to be complete; in the latter it is referred to as a mosaic mutation. Exposure of procaryotic cells as well as eucaryotic cells to mutagenic agents usually gives both kinds of mutation. However, in a few experiments with *Escherichia coli* (*1*) the mutations seemed to be complete. In my experiments with a eucaryote no mosaic mutants were obtained.

The experimental object was the multicellular green alga *Ulva mutabilis* (*2*). Its life-cycle alternates between a haploid and a diploid phase, morphologically similar. The diploid sporophytes, developed from zygotes, form haploid zoospores by meiosis. The zoospores develop into haploid gametophytes which form haploid gametes through mitotic divisions. If the gametes are allowed to unite they form diploid zygotes; if not, the majority of them will develop parthenogenetically into haploid germlings which become diploid by chromosome doubling when they consist of more than 100 cells. The first cell division in a germling (gametophytic or sporophytic) occurs after 3 to 4 days of a development which starts when the reproductive units lose their motility.

The algae were grown in petri dishes with Erdschreiber medium (*3*) at 18°C at a diurnal cycle of 17 hours light and 7 hours dark. Zoospores—gametophytic germlings aged 0, 1, 2, 3, and 5 days respectively, swimming gametes, and 5-day-old germlings developed parthenogenetically from gametes, were irradiated with a 30 W Hanovia Chromatolite ultraviolet-lamp (*4*) and kept in the dark for 24 hours after exposure, to prevent photoreactivation. The plants were examined 15 to 20 days after irradiation, and specimens which differed morphologically from the wild type were isolated as presumptive mutants. In *Ulva* gametophytes may produce gametes and sporophytes may produce zoospores from almost the whole thallus. Progeny from any part of the thallus may thereby be examined. Only plants with mutant progeny or plants where parts of the thallus produced mutant progeny were finally considered to be mutants (Table 1). Mutants induced with ultraviolet irradiation were similar to spontaneous mutants collected during routine cultivation of the alga in the laboratory (*5*). All mutants, to date, show normal segregation.

Chimeras, which consist of two genetically different parts, developed among plants irradiated as 3- or 5-day-old germlings when the first cell divisions were observed in the cultures. These chimeras show that cells of different genotypes can express their own character phenotypically within the same plant. If mosaic mutations occur they could therefore result in chimeric plants and be detectable. However, chimeras were never detected among germlings irradiated at the single-cell stages (Table 1, *6*). Consequently, mosaic mutants do not occur or are extremely rare among mutants with a visible influence on the morphogenesis in *Ulva mutabilis*.

It is not known whether a eucaryotic chromosome is either structurally or functionally single- or multi-stranded, but even a single-stranded chromosome, as in the procaryotes, gives mosaic mutants, which is attributed to the

Table 1. Induction of mutants by ultraviolet irradiation in *Ulva mutabilis*. From random samples of approximately 150 plants it was estimated that 3-day-old zoospores have 1.34 cells per plant, 5-day-old zoospores have 4.88 cells per plant, and 5-day-old gametes have 4.27 cells per plant.

Survival range (%)	Age (days)	Plants examined (No.)	Mutants (No.)	
			Whole thallus	Chimeras
		Zoospores		
Control		12588	3	0
80–90	0	31601	79	0
50–60	0	10453	43	0
10–20	0	2242	21	0
80–90	1	43773	66	0
80–90	2	58425	61	0
80–90	3	63272	49	14
80–90	5	56426	12*	29
		Gametes		
Control		34139	6	0
80–90	0	18490	32	0
50–60	0	9520	48	0
10–20	0	8749	89	0
<2	0	1307	19	0
50–60	5	16382	4*	13

* Expected, according to the control, to be spontaneous mutants.

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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2. The species is morphologically similar to, but is sexually isolated from *U. lactuca* and *U. thureti*. B. Føyn, *Arch. Protistenk.* **102**, 473 (1958).
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.
5. About 200 spontaneous mutants have been found. Two of them were isolated as chimeras with the thallus consisting of a mutant and a wild-type part. B. Føyn, unpublished results. A few of the spontaneous mutants have been described: B. Føyn, *Arch. Protistenk.* **104**, 236 (1959); *Biol. Bull.* **118**, 407 (1960); *Bot. Marina* **3**, 60 (1961); *ibid.* **4**, 156 (1962).
6. This result indicates also that the genes were unduplicated at the time of irradiation. Since the 2-day-old gametophytic germlings are also included, the DNA replication may occur late in the cell cycle, as is the case in *Chlamydomonas reinhardi*, where chromosomal DNA replicates just before division. K. S. Chiang and N. Sueoka, *J. Cell. Physiol.* **70** (Suppl. 1), 89 (1967).
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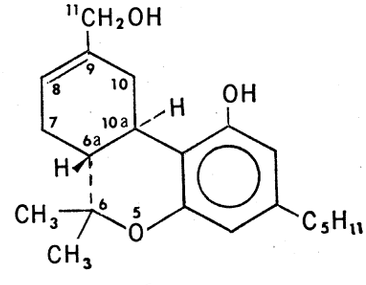
Metabolite of (−)-trans-Δ^8-Tetrahydrocannabinol: Identification and Synthesis

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.*

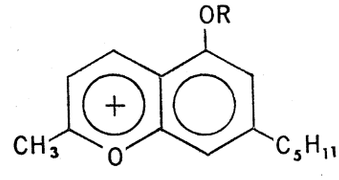
Increasing use of marijuana has generated interest in the metabolic fate of its psychotomimetically active constituents, (−)-*trans*-Δ^8-tetrahydrocannabinol (Δ^8-THC) and (−)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC) (*1*). While studying the distribution in the organs of rats after injection of ^{3}H-labeled 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