inhibition of *E. coli* DNA (*1*) despite the fact that the *E. coli* chromosome is about 20 times longer than the T4 DNA molecule. Whether the similarity is the accidental result of two different mechanisms or is due to similarity between the molecular structure of the replicating phage DNA and the bacterial chromosome, as the synthesis of DNA molecules with a higher molecular weight of the mature phages, remains undecided.

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References and Notes

1. O. Maaløe and P. C. Hanawalt, *J. Mol. Biol.* **3**, 144 (1961).
2. L. V. Crawford, *Virology* **7**, 359 (1959); R. Thomas, *ibid.* **9**, 275 (1959).
3. J. Tomizawa and S. Sunakawa, *J. Gen. Physiol.* **39**, 553 (1956).
4. A. D. Hershey and N. E. Melechen, *Virology* **3**, 207 (1957).
5. R. Sussman and F. Jacob, *C. R. Hebd. Seances Acad. Sci. Paris* **254**, 1517 (1962).
6. W. C. Schneider, *Methods Enzymol.* **3**, 680 (1957).
7. R. Favre, P. Amati, M. Bezdek, *Virology* **35**, 238 (1968).
8. I thank Prof. G. Streisinger, Dr. M. Terzi, and Prof. M. Fox for valuable discussions and Prof. S. E. Luria for help in the preparation of the manuscript. The technical assistance of L. Nielsen and A. Riccio is gratefully acknowledged.

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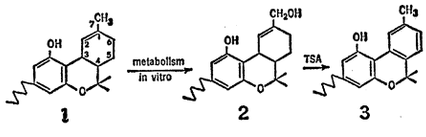
Δ^1-Tetrahydrocannabinol: Structure of a Major Metabolite

Abstract. *Δ^1-Tetrahydrocannabinol, the major psychotomimetically active compound of* Cannabis, *was metabolized in vitro by the 10,000g supernatant from rabbit liver. By mass and nuclear magnetic resonance spectrometry, the major metabolite was identified as 7-hydroxy-Δ^1-tetrahydrocannabinol. The latter compound of* Cannabis, *was metabolized in vitro by the 10,000g supernatant from*

Δ^1-Tetrahydrocannabinol (Δ^1-THC; **1**) is the major psychotomimetically active cannabinoid of *Cannabis*. Recently we described the rapid metabolism of Δ^1-THC in the rabbit (*1*) and found that at least three major metabolites are excreted in the urine. Using an in vitro technique we have now isolated and identified 7-hydroxy-Δ^1-THC (**2**) as a major metabolite of **1**.



1 **2** **3**

We used the in vitro system described by Tagg *et al.* (*2*) with some modifications. Four female rabbits given prior treatment with sodium phenobarbital were killed. The livers were homogenized with an equal volume of 1.15 percent KCl solution, and the homogenates were centrifuged for 10 minutes at 10,000*g*. The supernatant (500 ml) was incubated for 1 hour with Δ^1-[^{14}C]THC (*3*) (120 mg; 188 μc/mmole), nicotinamide (772 mg), nicotinamide-adenine dinucleotide phosphate (42 mg), glucose-6-phosphate (2864 mg), $MgCl_2 \cdot 6H_2O$ (1640 mg), and 0.06*M* phosphate buffer (200 ml, *p*H 7.0).

Extraction of the incubation mixtures with light petroleum removed most of the unchanged Δ^1-THC. The remaining radioactive material was extracted into diethylether. Thin-layer chromatography (*1*) of these extracts showed that 40 percent of the added Δ^1-THC had been converted to more polar compounds. In a control experiment with boiled supernatant Δ^1-[^{14}C]-THC was recovered unchanged.

The radioactive ether extract was chromatographed on a Florosil column (1.5 by 100 cm) and eluted first with mixtures of diethylether and light petroleum of increasing polarity and finally with pure ether. The remaining Δ^1-THC was found in the ether : light petroleum (1:5) fraction, and the metabolite was obtained from the pure ether fraction. Thin-layer chromatography (*4*) of the ether fraction showed a phenolic compound with an R_F of 0.5 containing 95 percent of the total radioactivity on the plate. This metabolite was further purified by preparative thin-layer chromatography (*4*) to yield 20 mg of a semicrystalline substance, which according to the specific activity was over 95 percent pure.

The nuclear magnetic resonance (NMR) spectrum (*5*) of the metabolite **2** was largely in agreement with that of Δ^1-THC with the following differences. The signal at $\delta = 1.69$ from the methyl group in position 1 of Δ^1-THC (*6*) was absent, and instead a two-proton signal at $\delta = 4.02$ ($-CH_2OH$) was recorded. Three one-proton signals of $\delta = 6.70$, 6.25, and 6.11 represented one vinylic and two aromatic protons.

The mass spectrum (*7*) showed a molecular ion at *m/e* (mass to charge) 330 (35 percent; Δ^1-THC + 16) and prominent peaks at *m/e* 315 (6 percent; $-CH_3$), *m/e* 312 (16 percent; $-H_2O$), *m/e* 300 (24 percent; $-CH_2O$), and *m/e* 299 (100 percent; $-CH_3O$). These data together with the information from the NMR spectrum indicated a hydroxylation only on the vinylic methyl group in position 1 of Δ^1-THC and no change in the aromatic moiety. This was further confirmed by the infrared (increased hydroxyl absorption) and ultraviolet spectra (maximum absorbance at 279 nm in ethanol) which were almost identical to the corresponding spectra of Δ^1-THC. These spectral data are consistent with the formulation of the metabolite as 7-hydroxy-Δ^1-THC (**2**).

Burstein *et al.* (*8*) reported the isolation in vivo and structure of a metabolite of the isomer $\Delta^{1(6)}$-THC in the rabbit. The metabolite was either 5-hydroxy, or more likely, 7-hydroxy-$\Delta^{1(6)}$-THC. In the latter case, the biochemical oxidation of Δ^1-THC and $\Delta^{1(6)}$-THC would be similar. Also this metabolite was dehydrated to cannabinol (**3**) with the use of *p*-toluenesulfonic acid (TSA) in refluxing benzene. With their procedure (*8*) we converted the in vitro metabolite **2** to the same compound **3**; the product was confirmed by thin-layer (*1*) and gas-liquid chromatography (5 percent XE-60; 5 percent SE-30 on Gas Chrom P).

Δ^1-Tetrahydrocannabinol seems to be excreted in completely metabolized form (*1*, *8*, *9*). The demonstration of 7-hydroxy-Δ^1-THC (**2**) as the first structurally known metabolite of Δ^1-THC (**1**) and the chemical conversion of the metabolite to the previously known cannabinol (**3**) could form the basis for an identification of *Cannabis* smokers. Experiments in mice have shown the metabolite to be pharmacologically strongly active.

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References and Notes

1. S. Agurell, I. M. Nilsson, A. Ohlsson, F. Sandberg, *Biochem. Pharmacol.*, in press.
2. J. Tagg, D. M. Yasuda, M. Tanabe, C. Mitoma, *ibid.* **16**, 143 (1967).
3. J. L. G. Nilsson, I. M. Nilsson, S. Agurell, *Acta Chem. Scand.* **23**, 2209 (1969).
4. Silica gel G, developed in diethylether. Spray reagent: 0.5 percent Echtblausalz (Merck) in 2*N* NaOH.
5. The NMR spectrum was obtained with a

Varian A60 spectrometer in $CDCl_3$ solution and with tetramethylsilane as internal standard.
6. T. Petrzilka and C. Sikemeier, *Helv. Chim. Acta* **50**, 2111 (1967).
7. Mass spectrum was obtained on an LKB 9000 mass spectrometer at 70 ev.
8. S. H. Burstein, F. Menezes, E. Williamson, R. Mechoulam, *Nature* **225**, 87 (1970).
9. G. Joachimoglu, J. Kiburis, C. Miras, *Prakt. Akad. Athenon* **70**, 161 (1967).
10. Part VII of "Metabolism of *Cannabis*." Supported by the Swedish Medical Research Council. We thank Dr. J. E. Lindgren for mass spectra, Dr. T. Petrzilka and R. Mechoulam for sharing information, and C. Enzell for helpful discussions.

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Aedes aegypti: Origin of a "New" Chromosome from a Double Translocation Heterozygote

Abstract. *An apparent enhancement of crossing-over occurs in the region common to two translocated chromosomes derived from two different reciprocal translocations in the yellow-fever mosquito,* Aedes aegypti. *A "new" chromosome containing parts of all three linkage groups is created through crossing-over. In a reversible process, two haploid translocated sets in the double translocation heterozygote produce a new, normal chromosome set and vice versa in alternating generations.*

Genetic control mechanisms for insect pests are currently being developed for a number of important species. To date, the most successful genetic mechanism has utilized the production of dominant lethal mutations by gamma radiation in males of the screw worm fly (*1*). Although sterilized males effectively reduce population size in the generation after their release, they do not convey sterility to members of the next generation; thus, continued reintroductions are necessary to maintain control.

The use of the semisterility inherent in some chromosomal aberration systems provides one means for introducing this inherited sterility into natural populations. The use of a reciprocal translocation system for insect control was originally postulated by Serebrovskii in 1940 (*2*). More recently Curtis (*3*) has indicated the potential effectiveness of translocation systems in insect control programs. In addition to the inherited semisterility, the translocation systems are usually associated with an essentially unaffected genotype and phenotype and may not therefore affect the competitive ability of released insects. Such reduced competitive ability often becomes a limiting factor in mutagen-induced dominant lethality. As a result, over the past few years several investigators have been interested in the use of chromosomal aberrations for the control of insect pests (*4*). Wagoner *et al.* (*5*) and Laven (*6*) have reported inducing several translocations in the house fly, *Musca domestica*, and the mosquito, *Culex pipiens*, respectively.

In our laboratory, we have been interested in inducing reciprocal translocations in the yellow-fever mosquito, *Aedes aegypti* ($2n = 6$) and evaluating the same for population control purposes. We have recently completed genetic analysis on two sex-linked translocations. One, RT(1:2) involves linkage groups one and two (*7*), with the original break points 0.3 crossover unit from the gene for sex (*M*) on group one and 1.6 crossover units from the wild type allele of spot abdomen (s^+) on group two. The other translocation, RT(1:3) involves linkage groups one and three, with the original break points 1.0 crossover unit from the wild type allele of red eye color (re^+) on group one and 0.5 crossover unit from the wild type allele of black tarsus (blt^+) on group three (*8*). It may be mentioned that sex in this species is determined by a single pair of alleles, *M* and *m*; the genotype *Mm* producing maleness and *mm* femaleness (*9*).

In an effort to determine the effectiveness of individuals carrying more than one translocation in reducing population sizes, a program was undertaken to produce individuals that would be heterozygous for both RT(1:2) and RT (1:3). Cytological analysis of these individuals would also provide information enabling the assignment of linkage groups to the individual chromosomes.

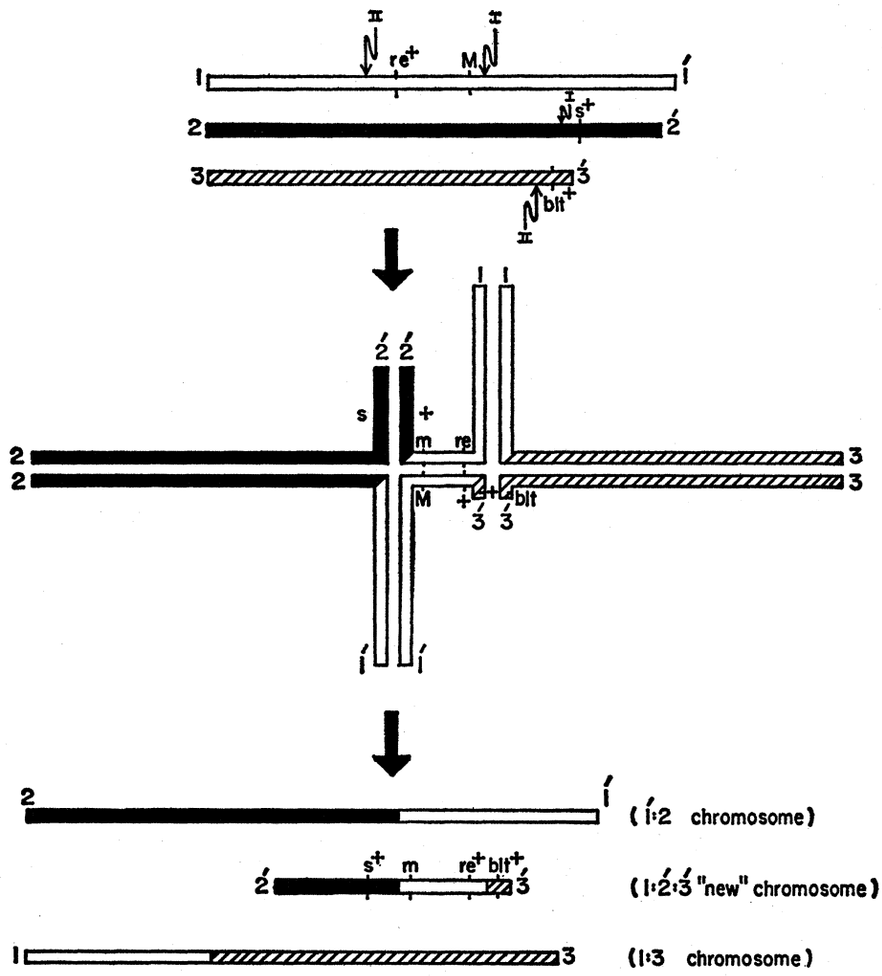

Fig. 1. Derivation of a "new" chromosome from a double translocation heterozygote. The three linkage groups (1-1́, 2-2́, 3-3́) are drawn to scale; the approximate location of the genetic markers used and the original radiation-induced breaks I and II producing RT(1 : 2) and RT(1 : 3), respectively, are indicated (top portion of the figure).