Table 2. Effects of juvenile hormone on cuticle deposition in cultured imaginal disks of *Plodia interpunctella*. The fat body, 10 percent ethanol, and dimethyl sulfoxide were present in each of these cultures.

| Donor larvae (mg) | Disks examined (No.) | 20-Hydroxy-ecdysone ($\mu$g/ml) | Juvenile hormone ($\mu$g/ml) | Disks with tanned cuticle (%) |
|---|---|---|---|---|
| 18-21 | 10 | 0 | 0 | 0 |
| 18-21 | 50 | 2.0 | 0 | 54.0 |
| 18-21 | 50 | 2.0 | 100.0 | 38.0 |
| 12-15 | 90 | 2.0 | 0 | 72.2 |
| 12-15 | 80 | 2.0 | 100.0 | 2.5 |
| 12-15 | 20 | 2.0 | 50.0 | 45.0 |
| 12-15 | 20 | 2.0 | 25.0 | 70.0 |
| 8-11 | 40 | 2.0 | 0 | 25.0 |
| 8-11 | 20 | 2.0 | 100.0 | 5.0 |

the development in vivo in which only the external surface of the pupal wing has tanned cuticle. Further comparisons with in vivo cuticle await a detailed histological study. However, as we know larval wing disks lack cuticle, the deposition of cuticle in vitro is by itself a suitable criterion of metamorphosis for this tissue.

We next determined whether or not juvenile hormone would prevent deposition of cuticle induced by 20-hydroxyecdysone in vitro. Juvenile hormone (100 $\mu$g/ml) caused a moderate inhibition of cuticle formation in disks from 18- to 21-mg larvae, and a pronounced inhibition in disks from 8- to 11-mg larvae. The most striking inhibition was observed with disks from 12- to 15-mg larvae. Only 2.5 percent of these disks made cuticle in response to 20-hydroxyecdysone at 2 $\mu$g/ml, fat body, and juvenile hormone at 100 $\mu$g/ml, compared to 72.2 percent with 20-hydroxyecdysone, dimethyl sulfoxide, and fat body. Dimethyl sulfoxide alone had no effect on disks cultured with fat body (Table 2). Disks cultured with juvenile hormone appeared healthy. Tracheal migration and elongation occurred even in disks in which cuticle deposition was prevented by the juvenile hormone.

Our observation that fat body potentiated the stimulation of cuticle deposition in vitro by 20-hydroxyecdysone in *Plodia* imaginal disks is of interest in view of the hypothesis that an interaction between fat body and $\alpha$-ecdysone may be important in the development of the wing disks of *Galleria* (*8*). Observations by Kambysellis and Williams (*11*) show that fat body may contain a macromolecular factor (MF) which promotes spermatogenesis in cultured *Cynthia* testes. As they also report that fetal calf serum, present in modified Grace's medium used in our cultures, contains MF, we believe that the fat body in our experiments supplied an additional factor or that it may have modified the 20-hydroxyecdysone molecule to a more active form.

Our results show that *Plodia* disks produced cuticle in response to 20-hydroxyecdysone in modified Grace's medium, although in an earlier experiment *Galleria* disks did not do so when cultured in chemically defined Grace's medium, which does not contain hemolymph or other serum (*7*). To test whether our new results were achieved by changing the medium or the species, we cultured disks from mature *Galleria* larvae in modified medium with fat body and 20-hydroxyecdysone. In these experiments, small patches of cuticle were observed in some disks, but there was not the extensive cuticle deposition seen in *Plodia* disks. The improved in vitro responses in cultured *Plodia* disks compared with the responses in *Galleria* disks were due to a change in species and perhaps, also, to a change in culture medium.

Our experiments in vitro suggest that, as the wing disks of the last larval instar of *Plodia* mature, they become less responsive to juvenile hormone but more responsive to 20-hydroxyecdysone. We believe that the inhibition in vitro of ecdysone-induced cuticle deposition may be an appropriate system for investigating juvenile hormone action.

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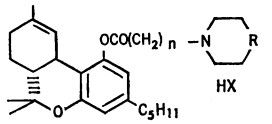
3 March 1972; revised 17 May 1972

## Water-Soluble Derivatives of $\Delta^1$-Tetrahydrocannabinol

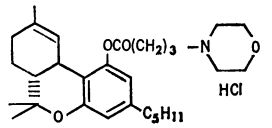
Abstract. *$\Delta^1$-Tetrahydrocannabinol, which is resinous and insoluble in water and therefore difficult to study pharmacologically, can be converted to a water-soluble derivative without loss of its biological activity. This has been achieved by preparing esters bearing a nitrogen moiety with the use of carbodiimide as the condensing agent. The availability of such water-soluble derivatives will allow the evaluation of $\Delta^1$-tetrahydrocannabinol in self-administration studies in monkeys for its addiction liability potential in man. This technique of water solubilization is also applicable to other compounds of chemical and biological significance.*

$\Delta^1$-Tetrahydrocannabinol ($\Delta^1$THC) is a resinous material which is insoluble in water and is administered in various solvents, such as polyethylene glycol, Tween, triton, and alcohol, which are not without pharmacological activity (*1*). Hence, the need for a water-soluble derivative of $\Delta^1$THC is apparent (*2*). The availability of such a derivative should facilitate pharmacological studies and allow evaluation in self-administration monkey studies (*3*). An ester derivative of $\Delta^1$THC is an obvious choice, but so far the conventional methods of esterification have not been successful.

We now report that water-soluble esters of $\Delta^1$THC of type **1** can easily be prepared with carbodiimide as the condensing agent. Thus compound **2** is a solid, freely soluble in water, and is quickly hydrolyzed to $\Delta^1$THC by liver




**1** R = O, $CH_2$

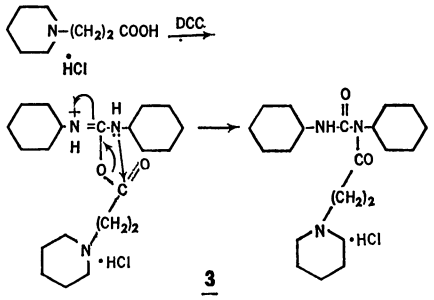



**2**

microsomal preparations. In the unanesthetized dog it causes ataxia, and the dose and the onset of action are comparable to those seen with $\Delta^1$THC.

Equimolar quantities of $\Delta^1$THC, dicyclohexylcarbodiimide (DCC), and $\gamma$-morpholinobutyric acid hydrochloride (*4*) in methylene chloride were stirred at room temperature for 16 hours. The dicyclohexylurea formed was filtered, and the methylene chloride was evaporated to leave a residue. The residue was triturated with ether to leave a solid (melting point 99° to 101°C); $[\alpha]_D$, −124.2°C (ethanol); homogeneous on thin-layer chromatography; nuclear magnetic resonance (NMR) ($CDCl_3$), showed $\delta$, 0.87 (3H, t, $\omega$-$CH_3$); $\delta$, 1.07, 1.38 (6H, 2s, *gem*-dimethyl); $\delta$, 1.67 (3H, s); $\delta$, 3.2 (6H, m); $\delta$, 4.1 (4H, m); $\delta$, 5.93 (1H, br, olefinic); $\delta$, 6.40, 6.58 (2H, 2d, $J = 3$ hz aromatic H); infrared ($cm^{-1}$, smear) 1756 (ester).

Similarly the piperidine derivative **1** (R = $CH_2$, n = 3) was prepared with the appropriate acid (*4*). However, when the $\beta$-piperidinopropionic acid hydrochloride (*4*) was used, the reaction in the presence of DCC took a different course (*5*), and only a very small quantity of the desired material was obtained by chromatographic separation




**3**

on Florisil. In this reaction most of the material isolated was identified as the *N*-acylurea **3**; NMR ($CDCl_3$) showed $\delta$, 1.0 to 2.1 (26H, m); $\delta$, 2.44 [4H, m, $N(CH_2-)_2$]; $\delta$, 2.66 (4H, m, $-CH_2-CH_2-$); $\delta$, 3.84 (2H, m, CHR'-RN–); $\delta$, 7.82 (1H, d, NH, $D_2O$ exchangeable); infrared ($cm^{-1}$, smear) 1700, 1625. Satisfactory elemental analyses were obtained for all new compounds. The reaction of carbodiimides with acids to give *N*-acylurea is known (*6*).

Table 1. Effect of compound **2** given intravenously to dogs. This scale for determining the effects of cannabinoid drugs in dogs is that used by Walton *et al.* (*13*) as modified by Dewey *et al.* (*14*). The range is from 0 or no effect to 6 when the dog is unable to stand without support. Ratings of 1 to 5 are based on the degree of ataxia and the severity of other cannabinoid symptoms.

| Dose (mg/kg) | No. | Effect | Rating |
|---|---|---|---|
| | | *Compound* **2** | |
| 0.5 | 2 | No change in behavior | 0 |
| 1.0 | 3 | Ataxia and depression | 1 |
| 2.0 | 1 | Drowsy-static ataxia—long duration | 2 |
| 5.0 | 2 | Ataxia, difficulty standing, salivation | 5 |
| | | *$\Delta^1$THC* | |
| 0.2 | 4 | No change in behavior | 0 |
| 0.5 | 2 | Slight ataxia and hyperexcitability | 1 |
| 1.0 | 2 | Drowsy-static ataxia—long duration | 2 |
| 2.0 | 2 | Ataxia and moderate depression | 4 |

When an aqueous solution of compound **2** was administered to dogs, its effects and duration of action were similar to molar equivalent doses of $\Delta^1$THC (Table 1). The onset of the characteristic ataxia in dogs with $\Delta^1$THC (*7*) and compound **2** was always within 10 minutes. In addition, compound **2** showed a similarity to $\Delta^1$THC in a number of other tests. For example, the median lethal dose ($LD_{50}$) of each was > 100 mg/kg, given intraperitoneally; and the median dose producing spontaneous activity ($ED_{50}$) was 7.6 mg/kg (range 3.9 to 14.8) for $\Delta^1$THC and it was 8.2 mg/kg (range 4.3 to 16.1) for compound **2** ($P < .05$). At 10 mg/kg given intraperitoneally, compound **2** decreased by 65 ± 8 percent the spontaneous activity in mice (*8*). Furthermore, like $\Delta^1$THC, it potentiated the hyperactivity induced by amphetamine (4 mg/kg given intraperitoneally) (*9*). At 40 mg/kg given intraperitoneally (equivalent to $\Delta^1$THC at 25.6 mg/kg) compound **2** caused an increase in activity in this test of 27 ± 4.5 percent, whereas at 25 mg/kg $\Delta^1$THC caused a 38 ± 6 percent increase.

A comparison of the preliminary cardiovascular studies of compound **2** and $\Delta^1$THC was made (Table 2). These studies were carried out in anesthetized dogs with the technique described by Dewey *et al.* (*7*). Both compounds caused a depression of blood pressure, and while the effect of compound **2** was less than with $\Delta^1$THC, the data were not significantly different. In four dogs, intravenous doses of compound **2** at 3.0 mg/kg caused a potentiation of the pressor responses that were due to epinephrine and norepinephrine. Simi-

Table 2. Cardiovascular response to $\Delta^1$THC and compound **2**. Four dogs were used in each determination. The dose of $\Delta^1$THC was 2 mg/kg, given intravenously. The dose of compound **2** was 3 mg/kg, given intravenously; PR, pressor response.

| Treatment | Response | *P* |
|---|---|---|
| Change in mean arterial blood pressure (mm-Hg) | | |
| $\Delta^1$THC | −34.0 ± 5.2 | |
| Compound **2** | −18.0 ± 6.5 | < .2 |
| PR to 2 $\mu$g/kg of epinephrine (mm-Hg) | | |
| Before $\Delta^1$THC | 43 ± 5.2 | |
| After $\Delta^1$THC | 73 ± 11.3 | < .05 |
| Before **2** | 22 ± 2.3 | |
| After **2** | 38 ± 4.3 | < .02 |
| PR to 2 $\mu$g/kg of norepinephrine (mm-Hg) | | |
| Before $\Delta^1$THC | 43 ± 5.3 | |
| After $\Delta^1$THC | 78 ± 9.2 | < .02 |
| Before **2** | 26 ± 5.8 | |
| After **2** | 42 ± 8.9 | < .1 |
| Change in duration of PR (%) | | |
| Epinephrine after $\Delta^1$THC | +32.6 ± 3.1 | |
| Epinephrine after **2** | +21.9 ± 3.0 | < .1 |
| Norepinephrine after $\Delta^1$THC | +36.5 ± 3.5 | |
| Norepinephrine after **2** | +28.7 ± 2.8 | < .3 |

lar effects were observed after the administration of intravenous doses of $\Delta^1$THC at 2 mg/kg.

Hydrolysis in vitro with microsomal preparations showed that compound **2** is quickly hydrolyzed to $\Delta^1$THC; $K_m$ (Michaelis constant), $6.3 \times 10^{-4} M$; $V_{max}$, $3.8 \times 10^{-8}$ mole/min per milligram of protein (*10*).

We had synthesized (*11*) another class of water-soluble esters of THC's (that is, the diethylaminobutyric ester) (*12*), which produced ataxia in unanesthetized dogs, similar to $\Delta^1$THC, except that the onset of action was considerably delayed and the effective dosage was five to ten times higher. In compound **2** these drawbacks have been eliminated, and it has a pharmacological profile similar to that of $\Delta^1$THC. It appears that the principal activity of compound **2** is due to hydrolysis in vivo to $\Delta^1$THC.

Our method of producing a water-soluble ester derivative of $\Delta^1$THC has also been applied to other compounds.

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## Superior Colliculus of the Tree Shrew: A Structural and Functional Subdivision into Superficial and Deep Layers

Abstract. *Superficial lesions of the superior colliculus produced deficits in form discrimination, while deeper lesions produced, in addition, an inability to track objects. These two syndromes were related to an anatomical subdivision: Superficial lesions resulted in anterograde degeneration in the visual thalamus, whereas lesions confined to the deeper layers produced degeneration in the nonvisual thalamus and in brainstem motor areas.*

The tectum appears to be the dominant visual center in nonmammals, but in mammals it is overshadowed by the geniculostriate system. These phylogenetic facts, along with data from the neurological clinic, led to the traditional view that the tectum serves only reflex functions, primarily in the coordination of head and eye movements (*1*). Sherrington's ideas provided an underlying support for this traditional view of the superior colliculus since, according to Sherrington, essentially all the central nervous system except for the cerebral cortex was involved in reflex functions (*2*). However, more recent studies, in which the ablation technique was used, suggest a more complex role for the tectum (*3–5*).

Our own interest in the tectum grew out of a comparative inquiry into the mammalian striate cortex, which revealed a surprising degree of preservation of form vision after removal of area 17 (*6*). These results turned our attention to the extrastriate visual system: the tectum and its cortical target, which is reached by a relay in the pulvinar (*7*). As a result of our comparative inquiry, we realized that it is an oversimplification to say that the tectum is progressively overshadowed by the geniculostriate system. Even among closely related species the size of the superior colliculus can vary considerably, in correlation with different ecological requirements.

In this report we provide evidence that the large and well-developed superior colliculus of the tree shrew (*Tupaia glis*) plays an important part in pattern vision: In particular, no animal with bilateral ablation of the superior colliculus learned to discriminate between an inverted and an upright triangle. In contrast, this task is easy for tree shrews after total removal of area 17 (*6*). A second finding was unrelated to the original intent of the experiment. We found that after shallow lesions of the superior colliculus the tree shrews exhibited normal cage behavior, while after deep lesions the tree shrews sat motionless in their home cages and appeared to be blind; they did not even withdraw from a threatening gesture. This difference in syndromes might be trivial if the milder one was simply the result of an incomplete lesion. However, we will also offer anatomical evidence which suggests that there are two structural subdivisions of the superior colliculus as defined by their connections: a superficial one and a deeper one.

In all, we have complete behavioral data for eight tree shrews and histological data for four of these cases. The remaining four are alive and are subjects of experiments in progress. There is every reason to believe that the completed cases are representative, so we will devote the remainder of this report to presenting the results for these cases.

In all four cases the superior colliculus was removed in anesthetized neonates by aspiration; aseptic techniques were used. After a suitable period of maturation a striking behavioral difference appeared, dividing the four cases into two classes. In two of the four animals visually guided behavior, as revealed by tests in the home cage, such as tracking of a food reward, appeared normal. The other two usually remained motionless in their home cage, and this absence of behavioral response was especially striking when the animals were provoked by a threatening gesture. In