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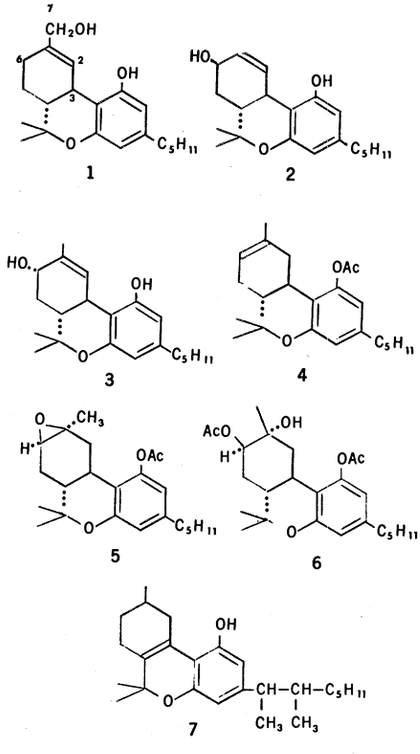
6β-Hydroxy-Δ¹-Tetrahydrocannabinol Synthesis and Biological Activity

Abstract. *6β-Hydroxy-Δ¹-tetrahydrocannabinol, a metabolite of Δ¹-tetrahydrocannabinol has been synthesized from Δ⁶-tetrahydrocannabinol. It shows high tetrahydrocannabinol-type activity in rhesus monkeys. The implications of this finding are discussed.*

The metabolism of Δ¹-tetrahydrocannabinol (Δ¹THC), the predominant active principle of marihuana (*1*), and of Δ⁶THC, a minor active component (*2*), has been extensively investigated (*3–5*). The major pathway so far identified is through hydroxylation of the allylic C-7 position of the terpenoid moiety. The metabolites 7-hydroxy-Δ¹-THC (**1**) and 7-hydroxy-Δ⁶THC (*6*), are biologically active, and it has been suggested (*7*) that these compounds are indeed the active forms of the respective THC's on the molecular level. Wall (*8*) has isolated a new metabolite, 6β-hydroxy-Δ¹THC (**2**), from an incubation of Δ¹THC with rabbit liver homogenate. The biological activity of **2** was not determined, apparently because of the difficulty of securing sufficient quantities for testing.

In continuation of our study (*9*) on structure and activity in the THC series we had already prepared both 6β-hydroxy-Δ¹THC (**2**) and 6α-hydroxy-Δ¹THC (**3**) when the identification of **2** as a metabolite was announced by Wall. For the synthesis of **2** we used Δ⁶THC acetate (**4**) as starting material. Epoxidation with *m*-chloroperbenzoic acid led to 1β,6β-epoxyhexahydrocannabinol acetate (**5**) in a yield of 84 percent. The molecular weight (372, determined from the mass spectrum) showed the addition of a single oxygen atom. The stereochemistry of the epoxy ring was determined from the nuclear magnetic resonance (NMR) spectrum: the signal of the equatorial C-6 proton at δ 2.87 appears as a doublet with a splitting constant of 4.5 hz. In the alternative axial position a large splitting constant of about 10 to 12 hz would have been expected.

Treatment of **5** with perchloric acid in acetone, followed by acetylation led to a mixture (*10*) which, on column chromatography over silica gel, gave, on elution with a 1 : 1 mixture of ether and petroleum ether, 1α-hydroxy-6β-acetoxyhexahydrocannabinol acetate (**6**) in 77 percent yield; the molecular weight was 432; $[\alpha]_D$ was −158° in ethanol; the ultraviolet spectrum showed a maximum λ_{max} in ethanol at 276 nm with molecular extinction, ε was 1770, and at 283 nm, ε was 1860. The NMR spectrum (*11*) gave evidence for six methyl groups [δ, 0.9 (t) 1.04, 1.12, 1.28, 2.00, 2.22 (singlets)], an equatorial proton in an α position to the acetoxyl group (δ, 4.76; bs, width at half height 4.5 hz), and two nonequivalent aromatic protons (δ, 6.21 and 6.40). Analysis of compound **6** showed



C, 69.49, H, 8.41; calculation for $C_{25}H_{36}O_6$ is C, 69.42; H, 9.15.

Dehydration of **6** by thionyl chloride in pyridine followed by reduction with lithium aluminum hydride led to a mixture (*10*) which was separated by preparative thin-layer chromatography giving 6β-hydroxy-Δ¹THC (**2**) in 24 percent yield. Compound **2** on analysis showed C, 76.09 and H, 9.03; calculation for $C_{21}H_{30}O_3$ indicates C, 76.33, H, 9.15; $[\alpha]_D$ was −133° in ethanol; ultraviolet spectrum, λ_{max} in ethanol, 276 nm (ε, 1510), 283 nm (ε, 1570); NMR in $CDCl_3$ (δ), 0.88 (t) side chain methyl; 1.07 (singlet), 1.38 (singlet) (methyl groups on C-8), 1.81 (singlet) (methyl group on double bond); 3.05 (bd, 10 hz) (C-3 proton); 4.05 (bd, 3.5 hz) (proton α to hydroxyl group); 6.06, 6.20 (aromatic protons); 6.65 (bs) (olefinic proton). The NMR spectrum is essentially identical to that reported by Wall (*8*) for the natural metabolite. Compound **2** is an unstable oil which, however, forms a stable 1 : 1 complex with dimethylformamide, m.p. 99°C. The NMR spectrum of this complex represents a summation of the spectra of **2** and dimethylformamide (in a ratio of 1 : 1) except for the aromatic protons which now appear at 6.17 and 6.21.

The NMR spectrum of 6α-hydroxy-Δ¹THC (**3**) (*4*) differs from that of the 6β isomer. Thus in **3** the C-6 proton appears at δ, 4.32 (bt, 11 hz).

6β-Hydroxy-Δ¹THC (**2**) was administered intravenously to rhesus monkeys in propylene glycol (0.1 ml per kilogram of body weight). The experimental conditions of this test have been described (*9*, *12*). At a dose of 1 mg/kg the monkeys were drowsy, showed significantly decreased motor activity, and occasional partial ptosis and head drop. These effects are identical to those observed with 0.25 mg of Δ⁶THC per kilogram of body weight in the same test. At a lower dose (0.5 mg/kg) 6β-hydroxy-Δ¹THC did not cause any observable effects in rhesus monkeys, while at a higher dose (2 mg/kg) we observed stupor, ataxia, suppression of motor activity, and full ptosis. The animals took up a typical crouched posture in which they remained for as long as 3 hours. The animals could, however, regain normal behavior for short periods of time if they were pinched or if they were presented with noise.

The isomeric 6α-hydroxy-Δ¹THC (**3**) is also active in rhesus monkeys at the same dose levels.

The above reported activity of 6β-hydroxy-Δ¹THC may complicate still further the quest toward the understanding of the molecular basis of *Cannabis* activity. Possibly Δ¹THC is likewise metabolized by humans to two (or more) active metabolites. This stipulated parallelism to rabbit liver homogenate metabolism, though plausible, has yet to be confirmed. If this is indeed the case, it will be of considerable importance to determine whether the nonidentical effects of *Cannabis* on different individuals are due, in part at least, to different ratios of Δ¹THC and metabolites (or only metabolites) at the receptor sites. Possibly each of these compounds acts in a slightly different fashion. Such a phenomenon is to be expected. The few psychotomimetically active cannabinoids that have so far been administered to humans cause effects that are by no means identical to those of Δ¹THC (*13, 14*).

It is generally assumed that the nonidentical psychological reactions produced by *Cannabis* are due to the different personalities of the users and the varying environmental conditions. While these factors are probably of considerable importance, one has now to take into account the possibility that several compounds (in varying ratios and with presumably different biochemical profiles) contribute to the overall effects.

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10. Additional components of this mixture were also isolated, and will be described (R. Mechoulam, in preparation).
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13. V. Sim [in *Psychotomimetic Drugs*, D. H. Efron, Ed. (Raven Press, New York, 1970), p. 333] has reported the evaluation in humans of the Δ³THC dimethylheptyl homolog **7** (which is a mixture of eight stereoisomers) and several of its pure isomers. Significant differences were observed both in potency and in effect. Two of the pure isomers of **7** caused postural hypotension and hypothermia but no hallucinogenic effects at doses up to 10 μg/kg. In contrast the total mixture **7** at 10 to 20 μg/kg caused mydriasis, thirst, headache, tachycardia, increase in blood pressure, and colored visual hallucinations but no hypotension or hypothermia. Some of these effects, although reminiscent of, also differ from those produced by Δ¹THC (*14*). One of us (R.M.) has been told by experienced *Cannabis* users both in the United States and in Europe that the dimethylheptyl analog of Δ¹THC (from illicit preparations) causes effects considerably different from those of Δ¹THC. These effects were reported to be mostly unpleasant and very prolonged.
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Galactose Toxicity in the Chick: Hyperosmolality

Abstract. *Galactose-fed chicks have been found to develop severe hyperosmolar dehydration. Although biochemical abnormalities have been observed in the brain of the galactose-toxic chick, the observed physiologic alteration of serum osmolality could be the major factor responsible for the galactose toxicity syndrome in the chick.*

The ingestion of galactose by chicks results in a syndrome consisting of shivering, generalized motor seizures, debilitation, and eventual death. The etiology of this syndrome is an enigma, but it has attracted attention as an animal model for the study of biochemical alterations leading to the mental retardation that occurs in galactosemia. It is known that the Rhode Island Red is more sensitive to galactose feeding than the Leghorn (*1*) and that the female is more sensitive than the male. Paradoxically, estrogen increases, whereas testosterone decreases, resistance to galactose feedings. In addition, galactose sensitivity decreases as the age of the animal increases (*1*). The accumulation of galactose metabolites in the tissues has been reported (*2, 3*), despite the fact that the galactose-fed chick is capable of metabolizing significant quantities of galactose (*4*). The clinical manifestations and the evidence of accumulated metabolites cease within 24 hours after galactose is removed from the diet (*2*).

Thus far, investigation has focused on a biochemical explanation for these observations. While working with galactose-fed chicks, however, we were struck by the severity of the animals' debilitation. The experimental animals are lighter and less vigorous than age-matched controls; when killed, the experimental animal bleeds very little. We also have observed that the experimental animal excretes large amounts of watery waste material not seen in control animals. Since these results suggest alteration in body fluids and dehydration, the galactose-toxic chick was investigated for changes in serum osmolality.

One-day-old Rhode Island Red chicks were obtained from Hall Brothers' Hatchery, Wallingford, Connecticut. Control animals were fed a diet of nonmedicated chick feed and water ad libitum. Experimental animals were given 10 percent galactose (weight-volume) in their drinking water for 2 days prior to being killed. Glucose and galactose concentrations in blood and cloacal contents were determined on protein-free filtrates as the trimethylsilyl ether derivative by gas-liquid chromatography (*2*). Serum osmolality was determined on an osmometer (Fiske model 300).

One-day-old chicks fed 10 percent galactose in their drinking water for 2 days have large amounts of galactose in their blood and cloacal contents (Table 1). The serum osmolalities of these chicks are approximately 180 milliosmoles per kilogram greater than those of control chicks of the same age. Serum osmolalities 100 milliosmoles per kilogram greater than those of control animals persist up to 5 days of age. It is during this 3- to 5-day period when sensitivity to galactose appears to be greatest, and when the greatest percentage of fatalities occurs (*1, 2*).