to rigorous scientific criteria has been given here, is of such profound general importance as to merit further detailed investigation. Speculation as to the origin of such effects should be avoided.

* Acknowledgments. We thank Ms T. L. Pham and Ms K. Löppen for their technical assistance.

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0014-4754/88/090761-02$1.50 + 0.20/0

Enantiomeric cannabinoids: stereospecificity of psychotropic activity

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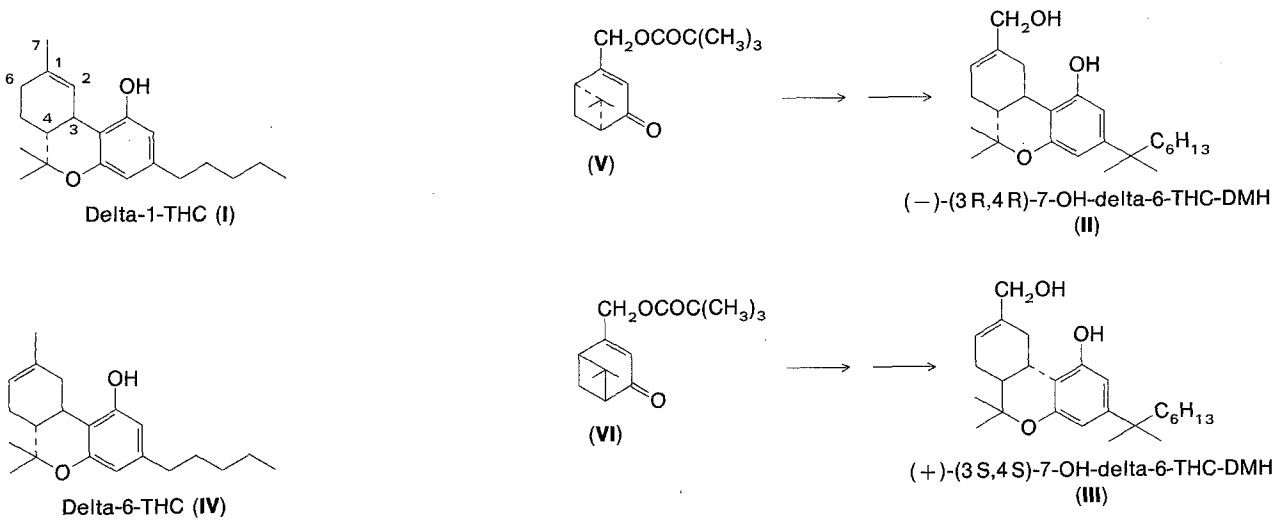
Received 3 March 1988; accepted 19 May 1988

Summary. The 1,1-dimethylheptyl homolog of (−)-(3R,4R)-7-hydroxy-delta-6-tetrahydrocannabinol (compound **II**) is highly psychotropic in mice, rats and pigeons. The (+)-(3S,4S) enantiomer (**III**) was found to be psychotropically inactive at doses up to several thousand times those of the ED_{50} of (**II**).
Key words. Cannabinoids; stereospecificity; psychotropic activity; drug discrimination.

The stereochemical requirements for cannabimimetic activity are not considered to be very high[3]. Cannabinoids with (3R,4R) configuration, which is the stereochemistry of natural delta-1-tetrahydrocannabinol (delta-1-THC) (**I**), are reported to be only about 10–20 times more active in tests for psychotropic activity than the corresponding synthetic (3S,4S) enantiomers. This presumed relatively low stereospecificity may have biological implications. Indeed, in a recent review[4] it was pointed out that 'while cannabinoid SAR supports the concept of a specific cannabinoid receptor, a disconcerting element is the apparent lack of greater stereoselectivity in some animal models.' However, most tests with synthetic (3S,4S) cannabinoids reported so far seem to have been performed with compounds of doubtful enantiomeric purity. Most, if not all (3S,4S) THC derivatives tested so far have been prepared following a route put forward by our group nearly 20 years ago which uses (+)-α-pinene as chiral starting material[5]. Commercial (+)-α-pinene is generally only 95–98% enantiomerically pure and would be expected to lead to products with a corresponding level of enantiomeric purity. Hence any psychotropic activity in the (3S,4S) series may have been due to the presence of variable amounts of the respective (3R,4R) enantiomer.

Materials and methods. The syntheses of compounds (**II**) and (**III**) will be reported separately. The starting material for (**II**) is enantiomerically pure 3-oxo-myrtenyl pivalate (**V**), m.p.



Structures of the natural tetrahydrocannabinols (delta-1-THC, **I**, and delta-6-THC, **IV**) and general indication of the synthetic route to the enantiomers of the 1,1-dimethylheptyl (DMH) homologs of 7-hydroxy-delta-6-tetrahydrocannabinol (compounds **II** and **III**).

Table 1. Generalization tests with various doses of (−)-delta-1-THC (**I**), (−)-7-OH-delta-6-THC-DMH (**II**) and (+)-7-OH-delta-6-THC-DMH (**III**) in rats and pigeons trained to discriminate between the presence and absence of the effect induced by delta-1-THC.*

Compound		n	Dose range (mg/kg)	Interval range (h)	ED_{50} (mg/kg)	r
delta-1-THC	(R)	14	0.30–5.6	0.5–6.5	0.85	0.94
	(P)	7	0.10–0.56	0.5–9.0	0.16	0.91
(−)-7-OH-delta-6-THC-DMH (**II**)	(R)	11	0.003–0.03	0.5–6.5	0.0098	0.99
	(P)	5–7	0.0001–0.0056	0.5–9.0	0.0022	0.99
(+)-7-OH-delta-6-THC-DMH (**III**)	(R)	4–9	3.0–10.0	0.5–6.5	> 10.0	–
	(P)	4	3.0–10.0	0.5–9.0	> 10.0	–

* The animals, rats (R) and pigeons (P), were trained to discriminate between delta-1-THC (3 mg/kg, rats; and 0.56 mg/kg, pigeons) and vehicle administered 0.5 h i.p. (rats) or 1.5 h i.m. (pigeons) prior to session onset. The ED_{50} values are based on the % RDP (percentage of responding to drug, THC, associated position) during the test probe yielding the highest % RDP, i.e. the lowest ED_{50} value indicating the peak effect. n, number of observations on which data points are based. ED_{50} values refer to logarithmic regression analysis; r, correlation coefficient for the regression. The data for delta-1-THC is from Järbe et al.[9].

40–42 °C, $[\alpha]_D$ − 158°; that for **III** is 3-oxo-myrtenyl pivalate (**VI**), m.p. 40–42 °C, $[\alpha]_D$ + 159°. Both enantiomers (**II**) and (**III**) melt at 140–141 °C; (**II**) has a rotation of $[\alpha]_D$ + 227° ($CHCl_3$) while (**III**) has a rotation of $[\alpha]_D$ − 226° ($CHCl_3$). These values were obtained after two recrystallizations of each cannabinoid. Enantiomers (**II**) and (**III**) have identical I.R. and N.M.R. spectra.

For discrimination testing, male rats (Alab AB, Sprague-Dawley, Sollentuna) and male pigeons (Palmetto White Carneaux, Sumter, S.C.) were trained to discriminate between delta-1-THC and vehicle [5% propylene glycol, 2% Tween-80 and 93% physiologic saline (v/v)], according to published procedures[6, 7]. The training doses of delta-1-THC were 3 mg/kg (2 ml/kg) or 0.56 mg/kg (1 ml/kg), administered intraperitoneally (i.p.) or intramuscularly (i.m.) 0.5 or 1.5 h prior to the onset of the training session in rats and pigeons, respectively. The experimental chambers, adapted after Ferster and Skinner[8], contained two response manipulanda separated by a recess in which liquid rewards (4-s access to a 0.1% saccharin/water solution) for rats or grain rewards (4-s access to chicken pellets) for pigeons, could be presented. Beforehand the rats and pigeons had been deprived of water and food, respectively. Which manipulandum was correct in a given session depended upon whether delta-1-THC or vehicle had been administered prior to training. Responses on the inappropriate manipulandum were recorded but had no programmed consequences. Fixed ratio schedules of reinforcement were used (FR-10, rats, and FR-15, pigeons), i.e. the reward was delivered when 10 (rats) or 15 (pigeons) responses had been accumulated on the state appropriate manipulandum. The rats were trained in 15-min sessions, 5 days a week, and the pigeons in 20-min sessions, 3 days a week. In each stimulus generalization test session a 'repeated tests procedure' was employed[6, 7], meaning that the choice behavior of the animals was evaluated several times after a single administration of a given dose of the drug during the particular test day (once or twice a week for rats and only once for pigeons); this test procedure has been described in detail elsewhere[9].

For the rat rotarod (ROT) test[10], male 150–200-g rats (Harlan Sprague Dawley, Indianapolis) were used. The cannabinoids were dissolved in polysorbate 80 and then distilled water was added so that the ratio of solvents in the final solution was 1:9 (v/v). The rats were injected i.v. and were tested 15 min later, a peak-effect time. Effects were measured as the ability or inability (neurotoxicity) of trained rats to remain on a revolving rotorod for 60 s. Data from separate groups of 10–15 rats per dose and 3–6 doses per cannabinoid were used to calculate median neurotoxic (TD_{50}) doses and related parameters[11].

For the mouse ring immobility assay[12] Sabra strain male mice (20–30 g) (Hebrew University Animal House, Jerusalem) were used. The cannabinoid was dissolved in ethanol and Emulphor 620 and then doubly distilled water was added. The ratio of the solvents (v/v) in the final solution was 5:5:90; injections were i.p. The assay measures the percentage of time spent immobile on a horizontal ring of 5.5 cm diameter during a 5-min exposure.

Table 2. Dose-response parameters of the enantiomers (**II**) and (**III**) in the rotarod (ROT) neurotoxicity test in rats*

Drug	ROT-TD_{50} (95% CL)
(−)-(3R,4R)-7-OH-delta-6-THC-DMH (**II**)	0.007 (0.005–0.009)
(+)-(3S,4S)-7-OH-delta-6-THC-DMH (**III**)	Not active (up to 15 mg/kg)
delta-6-THC (**IV**)	1.85 (0.98–3.45)

* Median toxic (TD_{50}) doses and 95% confidence limits (CL) are in mg/kg.

Table 3. Psychotropic effects of delta-6-THC (**IV**), (−)-7-OH-delta-6-THC-DMH (**II**) and (+)-7-OH-delta-6-THC-DMH (**III**) as evaluated by the mouse ring test.*

Dose (mg/kg)			
Vehicle	Delta-6-THC (**IV**)	(+)-7-OH-delta-6-THC-DMH (**III**)	(−)-7-OH-delta-6-THC-DMH (**II**)
	12.3 ± 2.0 (10)	12.0 ± 3.3 (10)	13.3 ± 2.8 (10)
10	41.3 ± 5.6 (7)	12.0 ± 3.3 (6)	–
5	–	14.2 ± 2.6 (6)	–
1	20.9 ± 1.6 (7)	13.8 ± 2.4 (6)	88.1 ± 2.3 (7)
0.5	–	11.6 ± 5.7 (5)	–
0.1	13.2 ± 1.8 (7)	–	55.1 ± 3.8 (7)
0.05	–	9.2 ± 2.1 (5)	–
0.01	–	–	24.9 ± 6.5 (7)
0.001	–	–	11.0 ± 1.8 (7)

* Numbers indicate percentage of time over 5 min a mouse stays immobile on a ring[11]. Numbers in parentheses indicate numbers of animals. Vehicle consists of ethanol: emulphor 620: water (1:1:18).

Results. In the drug stimulus generalization tests, presented in table 1, we found that the (−)-enantiomer (**II**) was ca 87 times more active than natural delta-1-THC (**I**) in the rat, and ca 73 times more active in the pigeon. The (+)-enantiomer (**III**) was inactive at doses ca 1000 times and ca 4500 times (for rats and pigeons respectively) higher than those of the ED_{50} of the (−)-enantiomer (**II**).

The same type of results were observed in the rotarod test in rats, presented in table 2. The (−)-enantiomer (**II**) was ca 260 times more potent than natural delta-6-THC (**IV**); the (+)-enantiomer (**III**) was inactive at doses ca 2000 times higher than those of the ED_{50} of the (−)-enantiomer (**II**).

Qualitatively the same type of results were obtained in the mouse ring test (see table 3): the (+)-enantiomer (**III**) was inactive in all doses tested; the (−)-enantiomer (**II**) was several hundred times more active than natural delta-6-THC (**IV**).

Discussion. The above results show clearly that in the enantiomeric pair of THC-type compounds (**II**) and (**III**) psychotropic activity resides solely in the (−)-(3R,4R) enantiomer (**II**), the (+)-(3S,4S) being inactive at doses up to several thousand times higher than the enantiomer ED_{50} of the (−)-(3R,4R) enantiomer (**II**). As mentioned above, this is in contrast to results obtained with other THC-type enantiomeric pairs [3]. As we believe that these differences are due to the presence of impurities of (3R,4R) enantiomers in the (3S,4S) enantiomers tested up till now, we are at present looking at synthetic routes leading to these (3S,4S) enantiomers with absolute stereochemical purity. However, on the basis of well-established structure-activity relationships [13] our results are very likely to be of general value. One of the structural changes of the THC molecule introduced by us, namely the hydroxyl group at C-7, is known to retain or increase THC activity. 7-Hydroxy-delta-1-THC and 7-hydroxy-delta-6-THC are major active primary metabolites of delta-1-THC and delta-6-THC, respectively [14]. The second change, the replacement of the n-pentyl side chain with a 1,1-dimethyl heptyl side chain, is also known to cause an increase in biological activity in all THC-type compounds tested so far [13]. We do not expect that these two chemical modifications would cause a pattern of activity different from that observed in other cannabinoids.

The (−)-(3R,4R) enantiomer (**II**) is one of the most psychotropic THC-type compounds in rodents and in pigeons reported so far. The ED_{50} for psychotropic activity of delta-1-THC and delta-6-THC are known for rodents and pigeons as well as for humans [7, 8, 14, 15]. If the animal to human ratios of activity established for these THC's are also valid for compound (**II**), marijuana-type psychotropic effects with (**II**) in man will appear at total doses as low as 0.1–0.2 mg. The psychotropically inactive enantiomer (**III**) is analgetic and antiemetic. These observations will be reported separately.

1 We thank Dr A. Breuer and Mrs H. Amsalem for help with the syntheses. The research reported above was supported in Tucson by NIH grant NS 15441; in Uppsala by grants from the Swedish Medical Research Council (5757) and the Swedish Council for the Planning and Coordination of Research (84/208:2); in Jerusalem by the Szold Foundation.

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0014-4754/88/090762-03$1.50 + 0.20/0

Hypothalamo-hypophyseal-gonadal function in the rat following administration of the novel and selective D-1 agonist CY 208–243

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Received 6 May 1988; accepted 9 June 1988

Summary. The effects of the novel and selective dopamine D-1 agonist CY 208–243 on the rat hypothalamo-hypophyseal-gonadal (HHG) axis were studied. CY 208–243 did not modify the concentration of luteinizing hormone (LH) in serum from female or male rats, and had no effect upon opiate antagonist-induced stimulation of LH secretion in male rats. CY 208–243 did not inhibit ovulation in cycling female rats. Thus, D-1 receptor activation by systemic drug administration does not alter HHG function in rats.
Key words. Hypothalamo-hypophyseal-gonadal axis; dopamine D-1 neurotransmission.

Dopamine D-1 neurotransmitter systems have been implicated in the central regulation of gonadotrophic hormone secretion in the rat. D-1 receptors have been identified in the median eminence [1], and a correlation between hypothalamic dopamine-stimulated adenylate cyclase activity, which is taken as an index for selective D-1 activation, and gonadotrophin release, has been observed [2]. Recently, a discrete stimulatory effect of a selective D-1 agonist on LH release within the zona incerta (Z.I.) of the hypothalamus has been described [3].