Biochemical Pharmacology, Vol. 23, pp. 439–446. Pergamon Press, 1974. Printed in Great Britain.

RELATIVE PHARMACOLOGICAL POTENCY IN MICE OF OPTICAL ISOMERS OF Δ^1-TETRAHYDROCANNABINOL

GARETH JONES,* ROGER G. PERTWEE, EDWARD W. GILL and WILLIAM D. M. PATON

University Department of Pharmacology, South Parks Road, Oxford, England

INGER M. NILSSON, MARIANNE WIDMAN and STIG AGURELL

Faculty of Pharmacy, University of Uppsala, Box 6804, 113 86 Stockholm, Sweden

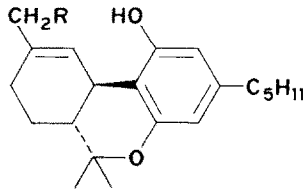
(*Received* 2 *April* 1973; *accepted* 10 *July* 1973)

Abstract—The tritium-labelled unnatural enantiomorph of Δ^1-tetrahydrocannabinol (Δ^1-THC) was synthesized. The ^{3}H-(+)-Δ^1-THC had a specific activity of 1·3 Ci/mmole and an optical purity of *ca.* 97%. The equipotent molar ratio for (+) and (−)-Δ^1-tetrahydrocannabinols was determined in mice by an established behavioural bioassay. The (+)-Δ^1-THC was found to be significantly less potent than the laevorotatory isomer, the mean potency ratio being 13 (95 per cent confidence limits: 7 and 24). Brain levels of (+)-Δ^1-THC and its metabolites were measured in mice 20 min after intravenous injection of ^{3}H-(+)-Δ^1-THC (2 mg/kg) and were compared with the corresponding levels of (−)-Δ^1-THC and its metabolites. With the exception of the concentrations of one metabolite, no statistically significant differences were observed between the mean levels of enantiomorphs of the cannabinoids in the brain. In the case of the single metabolite (which was tentatively assigned the structure of 7-hydroxy-Δ^1-THC) the brain level of the dextrorotatory isomer was 1·8-times higher than that of the laevorotatory isomer, a difference which was statistically significant. On incubation *in vitro* with an enriched mouse liver homogenate, (+)-Δ^1-THC was partially metabolized to more polar compounds; the principal metabolite was shown to be (+)-7-hydroxy-Δ^1-THC. It was concluded that the differences in the psychopharmacological potencies *in vivo* of the optical isomers of Δ^1-THC are determined within the central nervous system and are not due to gross differences in metabolism or body distribution.

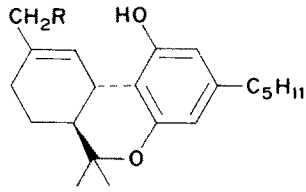
THE PSYCHOPHARMACOLOGICAL effects of cannabis are attributable chiefly[1–3] to Δ^1-*trans*-3,4-tetrahydrocannabinol (Δ^1-THC), a compound which is optically active and which exists naturally only in the laevorotatory form (Ia). Recently, Edery *et al.*[4] have found the synthetic enantiomorph, (+)-Δ^1-THC (IIa), to be at least 20-times less potent than (−)-Δ^1-THC in eliciting behavioural changes in rhesus monkeys.

(−)-Δ^1-THC is, however, rapidly metabolized in animals and man[5–7] to a complex mixture of products which includes (−)-7-hydroxy-Δ^1-THC (Ib). This major primary metabolite has been found to be strongly active pharmacologically[8–11] and to contribute significantly to the behavioural changes which are observed after administration of (−)-Δ^1-THC to mice.[12] The question therefore arises as to whether the difference in potency between optical isomers of Δ^1-THC is due to a true difference in the psychotropic activity of these molecules in the CNS, or to a major difference in the pattern of their metabolism or distribution in the body.

* Present address: Faculty of Pharmacy, Box 6804, 113 86 Stockholm, Sweden.



(I a) R = H
(I b) R = OH



(II a) R = H
(II b) R = OH

Formulae of (−)-Δ^1-THC (Ia), (+)-Δ^1-THC (IIa), (−)-7-hydroxy-Δ^1-THC (Ib) and (+)-7-hydroxy-Δ^1-THC (IIb).

To resolve this question we examined the relationship between the behaviour of mice and their brain levels of the Δ^1-THC enantimorphs and their respective metabolites. Similar experiments with (−)-Δ^1-THC and its major metabolite have shown a correlation to exist between behaviour and brain levels of active cannabinoids.[12,13]

MATERIALS AND METHODS

Drugs and reagents. Unlabelled (−)-Δ^1-THC was isolated from tincture of cannabis B.P.C.[14] and ^{3}H-(−)-Δ^1-THC (sp. act. 0·6 Ci mmole) was synthesized as described elsewhere.[15] (+)-Δ^1-THC was synthesized by the method of Mechoulam *et al.*[16,17] A mixture of *cis* and *trans* (+)-verbenols, $[\alpha]_{20}^{D} = +113°$ was prepared from (+)-α-pinene,* $[\alpha]_{20}^{D} = +47°$, according to the method of Whitham.[18] The mixture of (+)-verbenols was condensed with olivetol as described by Mechoulam *et al.*[16,17] to give (+)-Δ^6-THC, and this tetrahydrocannabinol was then isomerized to (+)-Δ^1-THC by the method of Petrzilka *et al.*[19] The product, $[\alpha]_{20}^{D} = +144°$, was obtained in an overall yield of 21 per cent from olivetol and was indistinguishable from natural (−)-Δ^1-THC by the criteria of TLC, GLC, i.r. and n.m.r. spectroscopy and mass spectrometry. For administration to mice, Δ^1-THC was dispersed with Tween 80 in 0·9% w/v aqueous sodium chloride. Δ^1-THC was injected into a lateral tail vein in a volume of 0·2 ml/25 g. The ambient temperature was 20–22°.

^{3}H-(+)-Δ^1-THC. ^{3}H-Dimethyl olivetol, specifically labelled in the 1′ and 2′ positions of the pentyl side-chain, was obtained essentially as described previously.[15] In the present synthesis, however, the starting material, 1-(3,5-dimethoxyphenyl)pent-1-ene, was catalytically reduced† in hexafluorobenzene over 10% palladium on charcoal using 100% tritium gas and the product was thus obtained with a specific activity of > 50 Ci/mmole. This high-activity ^{3}H-dimethyl olivetol was diluted with unlabelled material to give 5 mmoles at a specific activity of 1·3 Ci/mmole. After demethylation with boron tribromide, the resulting ^{3}H-olivetol was used in the preparation of ^{3}H-(+)-Δ^1-THC by a reaction sequence identical to that reported[15] for the synthesis of ^{3}H-(−)-Δ^1-THC except that (+)-verbenol was used in place of (−)-verbenol. The labelled product (sp. act. 1·3 Ci/mmole) was obtained in an overall yield of 11 per cent from the ^{3}H-dimethyl olivetol and was indistinguishable from the unlabelled (+)-Δ^1-THC by TLC, GLC and mass spectrometry. The ^{3}H-(+)-Δ^1-THC used in the present *in vivo*

* Generously donated by Bush Boake Allen Ltd.
† At the Radiochemical Centre, Amersham.

experiments was obtained after a further radiochemical dilution to a final specific activity of 0·6 Ci/mmole.

Radiochemical and optical purity of labelled tetrahydrocannabinols. Analysed by TLC and GLC, *ca.* 2% of the tritium in the ^{3}H-(−)-Δ¹-THC was present as ^{3}H-(−)-Δ⁶-THC, and *ca.* 3% of the label in the ^{3}H-(+)-Δ¹-THC was present as ^{3}H-cannabinol. Since the reagents and conditions employed in the synthesis of labelled and unlabelled (+)-Δ¹-THC were identical, optical data were obtained only for the unlabelled material and were assumed to apply to the tritiated product also.

The unlabelled (+)-Δ¹-THC, judged to be chemically pure by GLC, had $[\alpha]_{20}^{D} = +144°$ which (by calculation from the literature values[16,19] of −152° and −150·5° for optically pure (−)-Δ¹-THC) indicated an optical purity of *ca.* 97%. This was in good agreement with the optical purity of the (+)-α-pinene which was used as the starting material in the stereospecific synthesis; the pinene had $[\alpha]_{20}^{D} = +47°$, which (assuming a value of +51·5° for the pure enantiomorph[20]) indicated an optical purity of *ca.* 96%. Recently a high value (−163·4°) has been reported[21] for the specific rotation of (−)-Δ¹-THC, on the basis of which, the optical purity of our (+)-Δ¹-THC was *ca.* 94%. Although the specific rotations we observed for natural and synthetic (−)-Δ¹-THC's were similar to those reported by Mechoulam *et al.*[16] and Petrzilka *et al.*[19], there is obviously some doubt over the correct values for optically pure Δ¹-tetrahydrocannabinol enantiomers, and thus it is difficult to assess the level of contamination of our (+)-Δ¹-THC by the (−)-isomer.

Potency ratio of (+) and (−)-Δ¹-tetrahydrocannabinols in mice. The relative pharmacological potency of (+) and (−)-Δ¹-THC's in mice was determined by a 2 + 2 symmetrical dose assay[22] using the ring test.[23] The "immobility indices"[23] of male albino mice (23–27 g, Tuck strain No. 1) were determined about 1·5 hr before injection of either unlabelled (+)-Δ¹-THC (6·0 or 30·0 mg/kg) or unlabelled (−)-Δ¹-THC (0·4 or 2·0 mg/kg). The dose of the vehicle, Tween 80, was 60 mg/kg. The immobility indices of the mice were redetermined 15 min after injection of the cannabinoids and an analysis of variance was carried out upon the results.

Correlation of the behaviour of mice with brain levels of cannabinoids. The methods employed in this experiment have been reported in detail in earlier papers.[12,13] The immobility indices of male albino mice (23–27 g) were determined about 1·5 hr before injection of either (+) or (−) ^{3}H-Δ¹-THC (dose 2 mg/kg). The dose of Tween 80 was 10 mg/kg. Fifteen min after injection, the immobility index of each mouse was redetermined and the mouse was killed with carbon monoxide. Blood (0·5–1·0 ml) was then removed by intracardiac puncture. Individual whole brains were removed, weighed and then rinsed and homogenized in 0·1 M phosphate buffer (pH 7·4). After extraction of the homogenates with ethyl acetate, Δ¹-THC and its metabolites in the extracts were assayed by chromatography on silica-impregnated paper and liquid scintillation counting as reported previously.[12,13] Unless otherwise stated, differences between groups of data were evaluated by Student's *t*-test ($P > \text{or} < 0{\cdot}05$) and limits of error are expressed as the standard error of the means.

The in vitro *metabolism of (+)-Δ¹-THC.* (+)-Δ¹-THC was oxidized *in vitro* by a mouse liver preparation similar to the rat liver system reported previously.[12] 32·4 g of chopped liver (from 28 male albino mice, NMRI strain, 20–25 g) were homogenized in 250 ml of ice-cold 0·1 M phosphate buffer (pH 7·4) containing magnesium chloride (0·013 M), and the homogenate was centrifuged at 10,000 *g* for 10 min. To the

supernatant were added glucose-6-phosphate (770 mg), NADP (300 mg) and glucose-6-phosphate dehydrogenase (100 units) dissolved in 20 ml of phosphate buffer. ^{3}H-(+)-Δ^1-THC (50 mg, sp. act. 1·3 mCi/mmole) was dispersed ultrasonically with Tween 80 (100 mg) in 10 ml of phosphate buffer and the dispersion was incubated with the microsome preparation for 3 hr at 37°. The incubate was then extracted with petroleum spirit (b.p. 60 80°; 3 × 300 ml) followed by diethyl ether (3 × 300 ml). The analysis and purification of the two fractions were accomplished as described in Results.

RESULTS

Potency ratio of (+) and (−) Δ^1-tetrahydrocannabinols. The immobility indices of mice 15 min after intravenous injection of either (+)-Δ^1-THC (6·0 or 30·0 mg/kg) or (−)-Δ^1-THC (0·4 or 2·0 mg/kg) are shown in Fig. 1. The laevorotatory isomer was much more potent than the dextrorotatory isomer, the mean potency ratio being 13. An analysis of variance showed that (1) the slope of the two-dose response plots did not deviate significantly from parallelism ($P > 0·05$); and (2) for each optical isomer, the mean immobility index at high dosage was significantly greater than at low dosage ($P < 0·05$). In addition, Fisher's *F*-test showed that the variances of the four experimental points in the assay did not differ significantly from one another ($P > 0·05$). A symmetrical 2 + 2 dose analysis[22] showed the 95 per cent confidence limits on the potency ratio to lie at 7 and 24.

Mouse brain levels of enantiomorphic Δ^1-THC's and metabolites. The radioactivity present in the brains of mice 20 min after injection of either ^{3}H-(+)-Δ^1-THC or ^{3}H-(−)-Δ^1-THC was resolved into four components as described previously.[13] No qualitative difference was observed between the cannabinoids present in the brain after administration of (+)-Δ^1-THC and those present after administration of (−)-Δ^1-THC. Thus the chromatograms of the brain extracts obtained from mice injected with ^{3}H-(+)-Δ^1-THC showed peaks of radioactivity at the R_f-values of authentic (−)-Δ^1-THC, (−)-7-hydroxy-Δ^1-THC, and a third, uncharacterized, component termed the

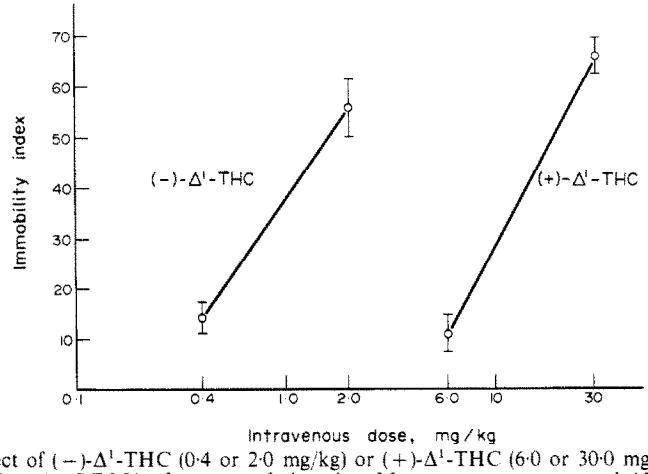


FIG. 1. The effect of (−)-Δ^1-THC (0·4 or 2·0 mg/kg) or (+)-Δ^1-THC (6·0 or 30·0 mg/kg) on the mean immobility indices (± S.E.M.) of groups of six mice. Measurement commenced 15 min after intravenous injection. The cannabinoids were dispersed in physiological saline by Tween 80 (dose in all cases: 60 mg/kg).

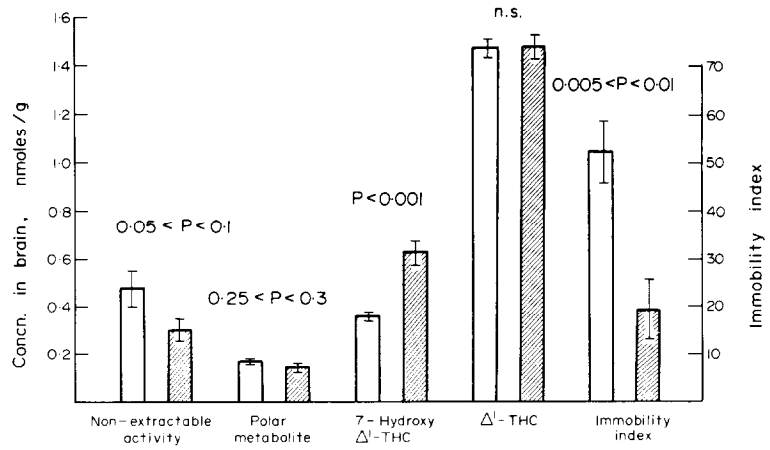


FIG. 2. Mean brain concentrations (± S.E.M.) of enantiomorphic Δ^1-THC's and their metabolites measured 20 min after intravenous administration to groups of six mice of either ^{3}H-(−)-Δ^1-THC (□, 2 mg/kg) or ^{3}H-(+)-Δ^1-THC (■, 2 mg/kg). The concentrations of cannabinoids are expressed in nmoles/g of wet brain tissue (left-hand ordinate). The mean immobility indices (± S.E.M.) measured immediately before determination of brain levels are also shown (right-hand ordinate).

"polar metabolite". A further fraction of the radioactivity in the brain was not extractable into ethyl acetate. The mean brain levels of the enantiomorphic forms of these four components are illustrated in Fig. 2. There was no significant difference between the two groups of mice in the mean total brain concentrations of Δ^1-THC and metabolites ($P > 0{\cdot}1$) and, with the exception of the concentrations of (+) and (−) "7-hydroxy-Δ^1-THC's", there was no significant difference in the brain levels of the individual enantiomorphic cannabinoids. The mean brain level of putative (+)-7-hydroxy-Δ^1-THC was, however, 1·8-times higher than that of the corresponding laevorotatory metabolite ($P < 0{\cdot}001$). The mean immobility indices of the groups following treatment with 2 mg/kg of (+) and (−) Δ^1-THC's were, respectively, 19 ± 6 and 52 ± 7. The difference between the indices was significant ($P < 0{\cdot}01$).

The in vitro *metabolism of* (+)-Δ^1-*THC*. Small aliquots of the petrol and ether extracts obtained after the *in vitro* incubation of ^{3}H-(+)-Δ^1-THC with the mouse liver preparation were chromatographed on Silica gel G (solvent: diethyl ether–petrol 7:3). The distribution of radioactivity on the chromatograms indicated that the cannabinoid content of the petrol fraction consisted essentially of unchanged (+)-Δ^1-THC (R_f 0·89) whereas the ether fraction contained a major metabolite (R_f 0·35) in addition to unchanged Δ^1-THC. The latter fraction was evaporated *in vacuo* at 30° and purified by column chromatography on Florisil (25 g), eluting initially with 20% diethyl ether in petroleum spirit (b.p. 60–80°) and then with 40% ether in petrol. Unmetabolized (+)-Δ^1-THC was eluted rapidly and was followed, considerably later, by the metabolite. Evaporation of the fractions containing the metabolite gave 25 mg of a residue which consisted of large quantities of non-radioactive lipoidal material in addition to the cannabinoid. The material was further purified by preparative TLC (fluorescent Silica gel G; ether–petrol 7:3) and the metabolite was eluted with diethyl ether (2 × 10 ml). Evaporation of the solvent gave 2·8 mg of metabolite which was, however, contaminated with Silica gel. Final purification was achieved by extraction with the n.m.r. solvent $CDCl_3$ followed by filtration.

The isolated material, which was *ca.* 90% pure by the criteria of TLC on alumina (solvent: 1% v/v methanol in chloroform) and GLC (2% SE 30 Ultraphase on Gas Chrom Q), showed the chromatographic mobility, colour reaction with Fast Blue B and retention time of authentic (−)-7-hydroxy-Δ^1-THC. The n.m.r. spectrum ($CDCl_3$: Varian A60 spectrometer) was almost indistinguishable from that reported[8] for (−)-7-hydroxy-Δ^1-THC; i.e. it was similar to that of (+)-Δ^1-THC except that the three-proton signal due to the methyl group at C_1 ($\delta = 1{\cdot}68$) was absent and there occurred instead a two-proton resonance ($-C\overset{*}{H}_2OH$) at $\delta = 4{\cdot}04$. The mass spectrum of the major component, obtained by GC MS on an LKB 9000 instrument, was essentially the same as that of (−)-7-hydroxy-Δ^1-THC: m/e 330 (20 per cent) 315 (5), 312 (17), 300 (30), 299 (100), 269 (13), 231 (27). The major *in vitro* metabolite of (+)-Δ^1-THC was thus identical with authentic (−)-7-hydroxy-Δ^1-THC by the criteria of TLC, GLC, n.m.r. and mass spectrometry and the former compound was therefore assigned the structure of (+)-7-hydroxy-Δ^1-THC (IIb).

A minor radioactive component (*ca.* 10% of the total), which was resolvable from (+)-7-hydroxy-Δ^1-THC by TLC and GLC on the systems described above, had a retention time and mass spectrum [m/e 330 (8 per cent), 312 (53), 297 (100), 296 (20), 295 (43), 229 (19), 214 (24)] quite similar to those obtained for (−)-6β-hydroxy-Δ^1-THC[5,24] under the same conditions. This component was, however, readily resolved from (−)-6β-hydroxy-Δ^1-THC by TLC on alumina and thus was shown not to be (+)-6β-hydroxy-Δ^1-THC. The compound may be a genuine *in vitro* metabolite of (+)-Δ^1-THC or, less probably, an artefact formed from (+)-7-hydroxy-Δ^1-THC. The occurrence of the molecular ion at m/e 330 suggests that this component is another mono-oxygenated derivative of Δ^1-THC. However the lack of sufficient material and the absence of appropriate reference compounds did not allow the identity of this minor component to be established.

DISCUSSION

Edery *et al.*[4] have reported that (−)-Δ^1-THC is considerably more potent than (+)-Δ^1-THC in producing behavioural changes in rhesus monkeys. The object of the present work was to test whether this difference in potency reflects a true difference in activity in the CNS between the two isomers, rather than lowered levels in the CNS of active (+) cannabinoids caused for example by a failure of the liver to form active (+) metabolites or by a particularly rapid degradation of the (+) compounds.

Using the mouse ring test, an assay with which limits of error can be obtained, we confirmed that (−)-Δ^1-THC was considerably more potent than the unnatural (+) isomer. The mean potency ratio was 13 (95 per cent confidence limits: 7–24). Since the (+)-Δ^1-THC which was used in the assay was not absolutely optically pure, these figures represent minimum values for the relative potency of the pure enantiomers. A major part of the apparent activity of the (+) isomer could be attributed to its small content (*ca.* 3%) of the (−) isomer.

The results obtained with the tritiated isomers further confirmed the higher activity of (−)-Δ^1-THC. The mean immobility index of the group of mice which had received ^{3}H-(−)-Δ^1-THC (2 mg/kg) was significantly higher than that of the group which had received ^{3}H-(+)-Δ^1-THC at the same dose. The mean total brain concentrations of cannabinoids, however, did not differ significantly. Further, with the exception of the concentration of the metabolite with the chromatographic properties of 7-hydroxy-Δ^1-

THC, there were no significant differences between the brain concentrations of individual cannabinoids. The mean brain concentration of putative (+)-7-hydroxy-Δ^1-THC in the group of mice treated with (+)-Δ^1-THC was 1·8-times higher than that of (−) metabolite in the second group. This difference could be due to: (1) a slightly faster hepatic monohydroxylation of (+)-Δ^1-THC relative to that of (−)-Δ^1-THC; or (2) slower metabolism of the (+) isomer beyond the initial monohydroxylation or (3) a more rapid transport of (+)-7-hydroxy-Δ^1-THC to the brain from the liver (and other sites of metabolism) perhaps as a result of different binding in the plasma.[25]

Though the major metabolite in the brains of mice injected with (+)-Δ^1-THC had the same chromatographic properties as (−)-7-hydroxy-Δ^1-THC, this alone was not conclusive evidence of its structure. Consequently, the metabolism of (+)-Δ^1-THC was investigated *in vitro* using an enriched mouse liver homogenate. The principal metabolite isolated from the preparation was shown to be (+)-7-hydroxy-Δ^1-THC by TLC, GLC, n.m.r. and mass spectrometry. This result indicates that the pattern of metabolism of (+)-Δ^1-THC by mouse liver *in vitro* is essentially the same as that[9] of (−)-Δ^1-THC, and supports the proposition that (+)-7-hydroxy-Δ^1-THC is the principal metabolite of (+)-Δ^1-THC in the mouse brain.

The results obtained *in vivo* with the tritiated Δ^1-THC enantiomorphs indicate, therefore, that the marked difference in the pharmacological potencies of these two isomers is not accounted for by a difference in amount of cannabinoids in the brain. The only detectable difference was in the brain levels of the isomeric 7-hydroxy-Δ^1-THC's. However, since (−)-7-hydroxy-Δ^1-THC is pharmacologically more potent in the mouse brain[12] than (−)-Δ^1-THC, and since the brain concentration of (+)-7-hydroxy-Δ^1-THC was higher than the corresponding concentration of (−)-7-hydroxy-Δ^1-THC, the difference in the levels of these metabolite enantiomorphs cannot explain, but rather accentuates, the difference observed in the *in vivo* activities of (+) and (−)-Δ^1-THC's.

It is concluded therefore that the difference in the pharmacological activities of the Δ^1-THC enantiomorphs in mice is attributable neither to major differences in the structures of their metabolites, nor to a failure of the dextrorotatory cannabinoids to appear in the brain. However, the difference in activity could be accounted for either by a difference in the pattern of distribution within the CNS or, alternatively, by a genuine difference in the pharmacological potency of the two isomers at their site of action. Since major differences in the brain distribution of these highly lipophilic and closely similar cannabinoids are unlikely, the second explanation would seem to be more plausible. These results thus support the hypothesis that cannabis activity is highly dependent on chemical structure and that a stereospecific molecular interaction is involved in its psychotropic action.

Acknowledgements We are indebted to Miss E. M. Spence and to Mrs. C. Stolt for technical assistance and to Drs. K. Leander and J.-E. Lindgren for n.m.r. and mass spectra. Reference 6β-hydroxy-Δ^1-THC was generously supplied by Prof. R. Mechoulam. The work was supported in part by the Medical Research Council and in part by the Swedish Medical Research Council. G.J. gratefully acknowledges the financial support of an M.R.C. Scholarship for Training in Research Methods and, latterly, of a Wellcome-Swedish Travelling Research Fellowship.

REFERENCES

1. Y. Grunfeld and H. Edery, *Psychopharmacologia* **14**, 200 (1969).
2. H. Isbell, C. W. Gorodetzsky, D. Jasinski, U. Claussen, F. v. Spulak and F. Korte, *Psychopharmacologia* **11**, 184 (1967).

3. R. MECHOULAM, A. SHANI, H. EDERY and Y. GRUNFELD, *Science* **169,** 611 (1970).
4. H. EDERY, Y. GRUNFELD, Z. BEN-ZVI and R. MECHOULAM, *Ann. N.Y. Acad. Sci.* **191,** 40 (1971).
5. M. E. WALL, *Ann. N.Y. Acad. Sci.* **191,** 23 (1971).
6. S. AGURELL, J. DAHMÉN, B. GUSTAFSSON, U.-B. JOHANSSON, K. LEANDER, I. M. NILSSON, J. L. G. NILSSON, M. NORDQVIST, C. H. RAMSAY, Å. RYRFELDT, F. SANDBERG and M. WIDMAN, in *Cannabis and its Derivatives* (Eds. W. D. M. PATON and J. CROWN) p. 16. Oxford University Press, London (1972).
7. L. LEMBERGER, *Adv. Pharmac. Chemother.* (in press).
8. I. M. NILSSON, S. AGURELL, J. L. G. NILSSON, A. OHLSSON, F. SANDBERG and M. WAHLQVIST, *Science* **168,** 1228 (1970).
9. H. D. CHRISTENSEN, R. I. FREUDENTHAL, J. T. GIDLEY, R. ROSENFELD, G. BOEGLI, L. TESTINO, D. R. BRINE, C. G. PITT and M. E. WALL, *Science*, **172,** 165 (1971).
10. L. LEMBERGER, R. E. CRABTREE and H. M. ROWE, *Science* **177,** 62 (1972).
11. M. PEREZ-REYES, M. C. TIMMONS, M. A. LIPTON, K. H. DAVIS and M. E. WALL, *Science* **177,** 633 (1972).
12. E. W. GILL, G. JONES and D. K. LAWRENCE, *Biochem. Pharmac.* **22,** 175 (1973).
13. E. W. GILL and G. JONES, *Biochem. Pharmac.* **21,** 2237 (1972).
14. E. W. GILL, *J. Chem. Soc.* **(C), 579** (1971).
15. E. W. GILL and G. JONES, *J. Labelled Comp.* **8,** 237 (1972).
16. R. MECHOULAM, P. BRAUN and Y. GAONI, *J. Am. chem. Soc.* **89,** 4552 (1967).
17. R. MECHOULAM, P. BRAUN and Y. GAONI, *J. Am. chem. Soc.* **94,** 6159 (1972).
18. G. H. WHITHAM, *J. Chem. Soc.* 2232 (1961).
19. T. PETRZILKA, W. HAEFLIGER and C. SIKEMEIER, *Helv. Chim. Acta* **52,** 1102 (1969).
20. Elsevier's Encyclopedia of Organic Chemistry, (Eds. E. JOSEPHY and F. RADT) Vol. 12A, p. 456 (1948).
21. A. A. LIEBMAN, D. H. MALAREK, A. M. DORSKY and H. H. KAEGI, *J. Labelled Comp.* **7,** 241 (1971).
22. D. COLQUHOUN in *Lectures in Biostatistics*, Chap. 13, Oxford University Press, London (1971).
23. R. G. PERTWEE, *Br. J. Pharmac.* **46,** 753 (1972).
24. Z. BEN-ZVI, R. MECHOULAM, H. EDERY and G. PORATH, *Science* **174,** 951 (1971).
25. M. WIDMAN, I. M. NILSSON, J. L. G. NILSSON, S. AGURELL, H. BORG and B. GRANSTRAND *J. Pharm. Pharmac.* **25,** 453 (1973).