Biochemical Pharmacology, Vol. 23, pp. 3017–3027. Pergamon Press, 1974. Printed in Great Britain.

BRAIN LEVELS AND RELATIVE POTENCY OF THE 1,2-DIMETHYLHEPTYL ANALOGUE OF Δ^1-TETRAHYDROCANNABINOL IN MICE

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(*Received* 1 *February* 1974; *accepted* 19 *March* 1974)

Abstract—Unlabelled and tritium-labelled (sp. act. 373 mCi/m-mole) forms of the 1,2-dimethylheptyl analogue of Δ^1-tetrahydrocannabinol (Δ^1-DMHP) were prepared as incompletely separated mixtures of threo- and erythro-isomers. The *n*-heptyl analogue (*n*-heptyl-Δ^1-THC), was also prepared, and this compound, and samples of Δ^1-DMHP containing different proportions of threo- and erythro-isomers, were compared with Δ^1-THC using the mouse ring "catalepsy" test. *n*-Heptyl-Δ^1-THC was found to be twice as active as Δ^1-THC (95 per cent confidence limits 1 and 3); the two isomers of Δ^1-DMHP were shown, within the limits of the assay, to possess equal activity, giving a mean potency ratio of 12. The duration of action of both drugs did not differ from that of Δ^1-THC. Brain and blood levels of Δ^1-DMHP and its metabolites were measured at various times after injection with ^{3}H-Δ^1-DMHP (0·1 mg/kg) and were compared with the corresponding levels of Δ^1-THC and its metabolites. The major metabolite in the mouse of Δ^1-DMHP was isolated from an *in vitro* mouse liver preparation, and was identified as 7-hydroxy-Δ^1-DMHP. The brain levels of this metabolite and Δ^1-DMHP itself correlated equally well with the behavioural effect, and showed that the higher activity of Δ^1-DMHP is not due to metabolic or distribution effects, as both the amount of 7-hydroxy metabolite relative to the parent, and that, in spite of its greater lipid solubility, the fraction of the injected dose which reached the brain were lower than for Δ^1-THC. It was concluded that the differences in the potencies of Δ^1-THC and Δ^1-DMHP in producing behavioural changes in the mouse are due to differences in activity at the site of action.

IT HAS long been known (see review by Mechoulam and Edery[1]) that small changes in chemical structure can produce large changes in the pharmacological potency of Δ^1-tetrahydrocannabinol (Δ^1-THC; I, R = C_5H_{11}). However, it is also known that Δ^1-THC is rapidly metabolized, and that at least one of its major metabolites (7-hydroxy-Δ^1-THC) makes a significant contribution to its activity in mice,[2] and probably also in man. Thus the caveat applied to most structure-action studies, that differences in potency might be due in part to differences in metabolism or distribution, applies with particular force in the tetrahydrocannabinol series. Recent experiments[3] with the "unnatural" (+) isomer of Δ^1-THC have demonstrated that the difference in potency between the two optical isomers does not arise from metabolic or distribution differences, and that the site of action is significantly asymmetric. As a continuation of these studies it was thought to be of interest to obtain a cannabinoid that was substantially more active than Δ^1-THC and to establish, by studying the tissue

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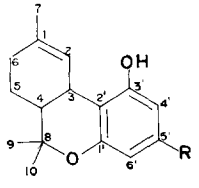
distribution of its tritium-labelled derivative, whether its activity was a consequence of molecular specificity at the CNS site of action. Early work by Adams *et al.*[4] on derivatives of Δ^3-THC indicated that replacing the pentyl side chain found in the naturally occurring cannabinoids with a 1,2-dimethylheptyl group resulted in a very considerable increase in activity and this observation has attracted a great deal of interest.[5] This paper describes results obtained with the tritium-labelled dimethylheptyl analogue of Δ^1-THC (Δ^1-DMHP,II).* Measurements were also made of the potency of the *n*-heptyl analogue.

Since this work was completed two other reports[1,6] on the activity of Δ^1-DMHP have been published.



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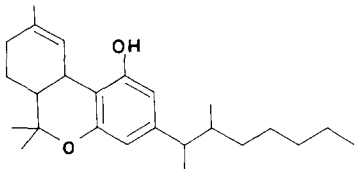

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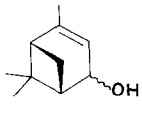

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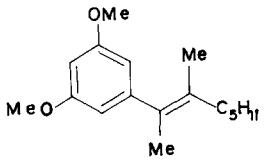

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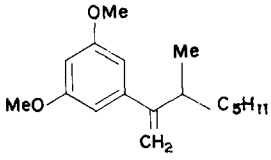

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MATERIALS AND METHODS

Drugs

The synthesis of ^{3}H-Δ^1-THC (sp. act. 571 mCi/m-mole) has been described elsewhere.[7]

Synthesis of tritium-labelled and unlabelled Δ^1-DMHP(II)

^{3}H-Δ^1-DMHP was prepared via the condensation of (−)-verbenol (III) with ^{3}H-5-(1,2-dimethylheptyl)-resorcinol, the label being located at the 1- and 2- positions of the side chain. Except for the synthesis of the resorcinol, all stages in the synthesis were carried out in a manner exactly analogous to that described[7] for Δ^1-THC. The synthesis was first carried out with the unlabelled material in order to confirm the structures of the products at each stage, and was then repeated exactly with the tritiated resorcinol. For the unlabelled material elemental analyses were taken of all the products and were all within $\pm 0{\cdot}3\%$ in carbon and hydrogen. The identities of important intermediates were also checked by infra-red, nuclear magnetic resonance and mass spectroscopy, and in all cases these agreed with available published data, and were consistent with the required structure. The purity of all products was con-

* The nomenclature of the cannabinoids is complicated by the lack of a trivial name for the tricyclic ring system, and analogues of THC with alkyl side chains other than pentyl have to be described by complex systematic names. One such systematic name for the ring system is tetrahydrodibenzpyran and, following Adams *et al.*,[4] DMHP is used as the abbreviation equivalent to THC.

firmed by gas–liquid chromatography (g.l.c.) and thin-layer chromatography (t.l.c.). The synthesis described here differs from that published previously[8] in the methods used to introduce the alkyl chain into the resorcinol nucleus, the modifications substantially improving the yield of dimethylheptylresorcinol.

3,5-*Dimethoxy-acetophenone.* Methyl lithium, prepared in the usual way from methyl iodide (45 g) and lithium (2·5 g) in dry ether (300 ml), was treated at 5° with a solution of *N,N*-diethyl-3,5-dimethoxybenzamide (40 g) in ether (600 ml). After the reaction mixture had been stirred at 5° and then at room temperature, each for 1 hr, saturated aqueous ammonium chloride solution (500 ml) was added. The product was isolated by extraction into ether and subsequent distillation *in vacuo*, to give the ketone (22 g, 60%) as a yellow oil which slowly crystallized (b.p. 150–154°/0·1 mm; Adams[8] gives b.p. 115–128°/0·3 mm, m.p. 43°).

2-(3,5-*Dimethoxyphenyl*)-3-*methyl*-2-*octene* (IV) *and* 2-(3,5-*dimethoxyphenyl*)-3-*methyl*-1-*octene* (V). To 2-heptyl magnesium bromide, prepared in the normal manner from 2-bromoheptane (72 g) and magnesium (10·6 g) in dry ether (200 ml), was added 3,5-dimethoxy-acetophenone (45 g) in dry ether (200 ml), while gently stirring the reaction mixture under reflux. Heating was continued for 2 hr on completion of the addition and, after washing the ethereal solution with 2 M hydrochloric acid, evaporation of the solvent yielded a mixture of the tertiary alcohol and the styrene derivatives (IV and V). Dehydration of the alcohol was carried to completion by heating the crude product with toluene-4-sulphonic acid (1 g) in benzene (100 ml), for 1 hr, in a water entrainer. The crude styrene, obtained by evaporation of the solvent, after washing the solution with 1 M sodium carbonate solution, was freed from the large amount of polar impurities by partition between petroleum spirit (b.p. 60–80°) and 20% v/v methanol in water. Distillation of the petroleum spirit fraction *in vacuo* yielded a product (9·7 g, 32%), b.p. 125–126°/0·3 mm, which was shown by g.l.c. to consist of two components, which were shown by MS and NMR to be the 1-octene (V) and the 2-octene (IV, *ca* 4:1): at 60 MHz in carbon tetrachloride: 1-octene τ 4·88 and 5·03 (C_1 protons) and τ 7·45 (C_3 proton), and 2-octene 7·89 (C_1 protons).

2-(3,5-*Dimethoxyphenyl*)-3-*methyl-octane incorporation of the tritium label.* The mixture of 1- and 2-octenes (IV and V) was hydrogenated over a palladium catalyst. The product was shown to consist of two components (*ca* 4:1), identified as the threo- and erythro-isomers (Fig. 1) previously reported.[9] Demethylation of these with boron tribromide yielded the desired dimethylheptyl-resorcinol. Repetition of this stage using a mixture of 2 per cent tritium in hydrogen as previously described[7] gave the corresponding labelled compound with the tritium incorporated at the 1- and 2- positions of the sidechain.

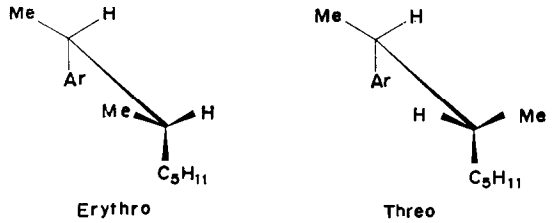

FIG. 1. Representation of the threo- and erythro-isomers of the 1,2-dimethylheptyl-resorcinol and Δ^1-DMHP (II): Ar signifies resorcinol or the dibenzpyranyl ring system.

Δ^1-DMHP and ^{3}H-Δ^1-DMHP. These compounds were obtained as previously described[7] for Δ^1-THC. The purified material was shown by g.l.c. to be present as two isomers, which could not be completely separated by column chromatography. Combined GC-MS of the unlabelled material showed that these two compounds had identical mass spectra, and were therefore identified as the threo- and erythro-isomers (Fig. 1), but no attempt was made to discover which was which. The product was obtained in three batches, the ratios of the isomers being: A 0·9:1; B 2·4:1; C 6·3:1, which were each stored at 250 μg/ml in dry benzene, under nitrogen. The specific activity of the labelled material was found to be 373 mCi/m-mole, and the radiochemical purity was shown, by g.l.c. and by paper chromatography followed by scintillation counting, to be at least 95 per cent. The octanol–water partition coefficient was determined as described previously.[10]

Synthesis of the n-*heptyl homologue of* Δ^1-*THC* (I, R = C_7H_{15})

This compound was prepared by exactly the same methods as were used[11] for the synthesis of propyl-Δ^1-THC, utilizing 3-heptyl-resorcinol obtained by the condensation of *n*-hexyl lithium with *N*,*N*-diethyl-3,5-dimethoxybenzamide.

Gas–liquid chromatography and mass spectrometry

All samples were analysed on a Pye 104 gas chromatograph with a column of 5% XE-60 on 80–100 mesh acid-washed Suprasorb, and an argon flow rate of 60 ml/min at temperatures between 200 and 250°, except for the combined GC-MS experiments, when a 2% SE-30 column was used with helium (40 ml/min) as the carrier gas. Samples obtained during metabolic studies were converted to their trimethylsilyl derivatives.[2] A manual preparative attachment allowed uncombusted samples to be taken of the components passing from the column for subsequent scintillation counting or mass spectrometry.

Mass spectra were obtained on an AEI MS9 instrument. In the combined GC-MS experiments the effluent gas from the g.l.c. was passed, via a gas separator, directly into the ionization chamber. Samples separated by the manual separator were dissolved in a small volume of ether and their spectra obtained in the normal way.

Liquid scintillation counting

Tritium was measured on a Beckman LS 200B instrument, with a scintillator solution[12] of PPO and POPOP in 2:1 v/v toluene: Triton X-100. Counting efficiencies were obtained using standard ^{3}H-hexadecane from the Radiochemical Centre, Amersham.

Bioassays

For administration to mice (male albino, 23–27 g, Tuck strain No. 1) cannabinoids were dispersed with Tween-80 in physiological saline; for experiments with unlabelled material the ratio by weight of Tween to cannabinoid was 2:1; for experiments with the labelled material the dose of Tween given was constant at 10 mg/kg. Injections were made into a lateral tail vein in a volume of 0·2 ml/25 g. Potency ratios were determined by a 2 + 2 symmetrical dose assay[13] using the ring test;[14] the doses used were 0·02 and 0·2 mg/kg (Δ^1-DMHP), 0·1 and 1·0 mg/kg (*n*-heptyl) and 0·2 and

2·0 mg/kg (Δ^1-THC). Immobility indices were determined about 1·5 hr before and 15 min after injection of the cannabinoids, using a double blind procedure.

Correlation of behaviour with brain levels of cannabinoids

At various intervals after injection (15, 90, 240 or 960 min) with Δ^1-DMHP (0·1 mg/kg), and 15 min after injection with ^{3}H-Δ^1-THC (2 mg/kg) the immobility index of each mouse was determined. The mouse was then killed with carbon monoxide and the blood and brain levels of the various cannabinoids and their metabolites were determined as previously described.[10] Whole brains were removed, washed and homogenized in 0·1 M phosphate buffer (pH 7·4); an aliquot of the homogenate was counted to determine the total radioactivity. The remainder of the homogenate was extracted with ethyl acetate and chromatographed on silica-impregnated paper to determine the amounts of radioactivity attributable to the parent compound and its metabolites. The solvent used for the chromatography of the extracts from mice treated with Δ^1-DMHP was 0·3% v/v methanol in chloroform. Whole blood was lysed with distilled water, extracted with ethyl acetate and chromatographed in a similar fashion. The blood from each mouse killed 20 min after injection with Δ^1-DMHP was collected as a separate sample; samples of blood collected from all other mice were pooled in groups of three. The immobility indices of mice, 90, 240 or 960 min after injection with Δ^1-THC were also determined. Differences between groups of data were evaluated using Student's *t*-test ($P >$ or $< 0{\cdot}05$). Limits of error are expressed as the standard error of the mean.

In vitro *metabolism of* Δ^1-*DMHP*

The *in vitro* metabolism was investigated by incubating Δ^1-DMHP (0·9:1 isomeric mixture; 10 mg) with a mouse liver preparation, in a manner similar to that described[2] for Δ^1-THC. Fresh, chopped mouse liver (10·1 g) was homogenized in ice-cold 0·1 M phosphate buffer (20 ml, pH 7·3) containing magnesium chloride (0·013 M), and the homogenate was centrifuged at 10,000 *g* for 10 min. The supernatant was divided into two portions of 8·8 ml, and to each was added 17·2 ml of the phosphate buffer and an additional 2 ml of buffer containing glucose-6-phosphate (68 mg), NADP (25 mg) and glucose-6-phosphate dehydrogenase (12·5 units). To one portion was added Δ^1-DMHP (10 mg) and to the other, Δ^1-THC (10 mg), each as a suspension with Tween 80 (20 mg) in saline (2 ml). The incubation mixtures were shaken for 3 hr at 37°, then extracted with ethyl acetate (3 × 20 ml). The amount of material extracted was estimated by g.l.c., by measuring the unchanged Δ^1-THC and Δ^1-DMHP. Half the extract from the Δ^1-DMHP incubation was purified by preparative chromatography (Whatman SG81 silica-impregnated paper; 0·3% v/v methanol in chloroform) and the component with the same R_f relative to Δ^1-DMHP as 7-hydroxy-Δ^1-THC has to Δ^1-THC was further purified on a second chromatograph. This material was converted to its trimethylsilyl ether,[2] when the major component (>90 per cent) was isolated by preparative g.l.c., and identified by mass spectroscopy.

RESULTS

Relative potencies of Δ^1-*THC analogues.* The immobility indices of mice 15 min after intravenous injection of various cannabinoids are shown in Table 1. Analysis

TABLE 1. THE RELATIVE POTENCIES OF ISOMERIC MIXTURES OF Δ^1-DMHP, Δ^1-THC AND THE *n*-HEPTYL ANALOGUE OF Δ^1THC, DETERMINED BY 2 + 2 SYMMETRICAL DOSE MOUSE IMMOBILITY INDEX ASSAYS (GROUPS OF SIX MICE).

Drug	Doses (mg/kg)	Mean immobility indices (± S.E.M.)		Molar potency ratio	95% Fiducial limits
		Low dose	High dose		
Δ^1-DMHP (0·9:1)*	0·02,0·2	21 ± 6	72 ± 2	15	—†
Δ^1-DMHP (6·3:1)*	0·02,0·2	16 ± 4	56 ± 8	9	3–25
Δ^1-THC	0·2,2·0	13 ± 6	67 ± 7	1	—
n-heptyl-Δ^1-THC	0·1,1·0	21 ± 4	73 ± 5	2	1–3
Δ^1-THC	0·2,2·0	23 ± 4	79 ± 7	1	—

* Mixture of threo- and erythro-isomers.

† Variance of one experimental point significantly greater than the variance of the other three points (F-test; $P < 0·05$).

of variance showed that for any single compound the responses at the two dose levels are significantly different, and that the slopes of the dose–response lines for different compounds do not differ significantly from one another. The *n*-heptyl analogue was found to be twice as active as Δ^1-THC, the 95 per cent confidence limits for the potency ratio lying at 1 and 3. The dimethylheptyl side-chain increases the number of possible isomers of the cannabinoid by virtue of its two chiral carbon atoms, and it was possible that these isomers, being diasteriomeric pairs, might not possess the same degree of activity. It was not possible to separate completely the Δ^1-DMHP into pure threo- and erythro-isomers, but comparison of the activity of two samples of the drug with different isomeric composition (0·9:1 and 6·3:1) against Δ^1-THC showed that, within the limits of the assay, the two isomers had the same potency: the molar potency ratios for the two mixtures relative to Δ^1-THC being 15 and 9 respectively (Table 1), giving a mean potency ratio of 12. As no significant difference in potency between the two isomers could be measured, the undifferentiated mixture of ^{3}H-Δ^1-DMHP was used for the tissue distribution studies. Using equipotent, submaximal doses, no difference in the durations of action of Δ^1-DMHP and Δ^1-THC could be detected.

In vitro *metabolism of* Δ^1*-DMHP*. The major *in vitro* metabolite of Δ^1-DMHP isolated after incubation with a mouse liver preparation was identified after purification, by the mass spectrum of its trimethylsilyl ether, as 7-hydroxy-Δ^1-DMHP. Major peaks in the mass spectrum were observed at *m/e* 530 (15 per cent), 459 (20), 431 (73), 430 (21), 427 (37), 359 (23), 341 (100), 284 (21). The parent ion (*m/e* 530) corresponds to a monohydroxy-derivative, and the strong peak at *m/e* 427 is attributed to the loss of a $[CH_2OSi(CH_3)_3]$ fragment which is consistent with hydroxylation at C7 but not at C6. The retro-Diels-Alder peak[15] at *m/e* 359 shows that hydroxylation has not occurred in the aromatic ring or on the alkyl side-chain. The yield of metabolite obtained by incubating Δ^1-DMHP with mouse liver homogenate was considerably lower than that obtained from Δ^1-THC. The total amount of material (Δ^1-DMHP and its metabolites) that could be extracted from the incubation mixture was considerably less than with Δ^1-THC, and it would appear that Δ^1-DMHP and its metabolites are much more strongly bound to tissue components. The overall yield of metabolite was only 4 per cent, and this was insufficient to obtain a reliable NMR spectrum.

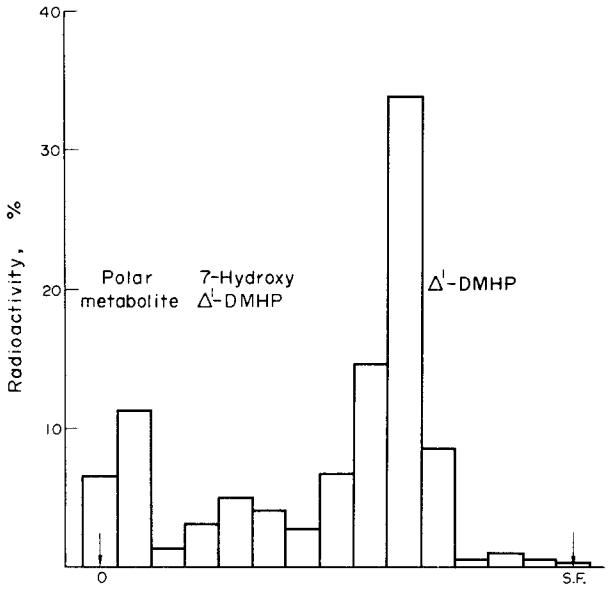

FIG. 2. Chromatogram of mouse brain extract 20 min after injection with ^{3}H-Δ^1-DMHP (0·1 mg/kg). The radioactivity in each strip is expressed as a percentage of the total extractable activity.

Brain levels of ^{3}H-Δ^1-DMHP and its metabolites. The radioactivity present in the blood and brains of mice up to 16 hr after injection with ^{3}H-Δ^1-DMHP was resolved into four major components in an exactly similar manner[10] to that after injection of Δ^1-THC (Fig. 2). The two most chromatographically mobile components had R_f values identical to those of Δ^1-DMHP and the 7-hydroxy-Δ^1-DMHP from the *in vitro* incubation. In addition, g.l.c. analysis of the trimethylsilyl ether of the component in brain extracts, of R_f identical to 7-hydroxy-Δ^1-DMHP, showed that about 90 per cent of the radioactivity flushed from the column emerged as a single peak with retention time identical to that of the metabolite obtained from the *in vitro* incubation. This component was assigned, therefore, to 7-hydroxy-Δ^1-DMHP, although small amounts of other metabolites may be present. The two other components were not investigated, and are subsequently referred to as "non-extractable material" and "polar metabolite".

Figure 3 shows the variation with time of the levels of the components identified as Δ^1-DMHP, 7-hydroxy-Δ^1-DMHP and polar metabolite in the brains of mice that had received Δ^1-DMHP (0·1 mg/kg). Also shown is the time course of the change in immobility index following injection of either Δ^1-DMHP (0·1 mg/kg) or Δ^1-THC (2·0 mg/kg). It will be seen that at the doses given, both the levels and the duration of the response are about the same for the two drugs. It will also be seen that as in the case[2,10] of Δ^1-THC significant amounts of Δ^1-DMHP metabolites are present in the brain 15 min after injection, and that brain levels of the parent compound and of the metabolites correlate equally well with the effect on immobility index. However, at any given time after injection, the molar ratio of metabolite to parent compound is less (1:10) in the case of Δ^1-DMHP than for Δ^1-THC (1:6).

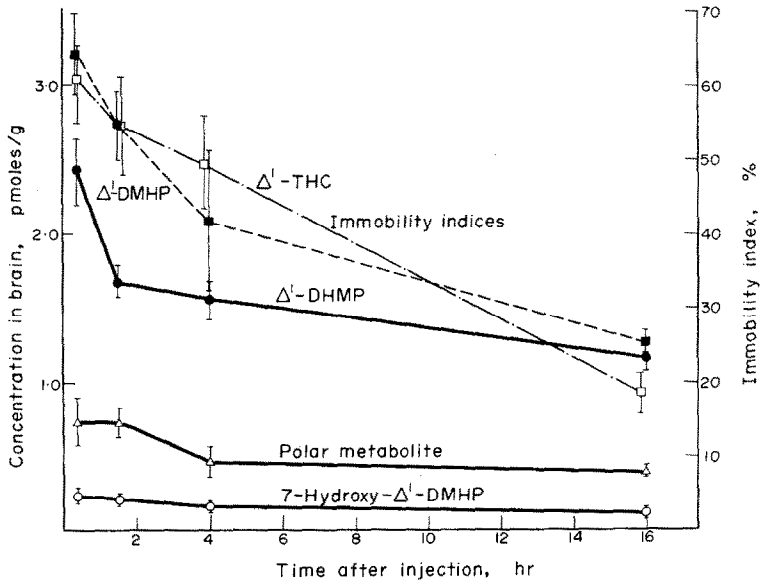

FIG. 3. Mouse brain concentrations of Δ^1-DMHP and its metabolites (solid lines: left hand ordinate) and immobility index (broken lines: right-hand ordinate), after injection of ^{3}H-Δ^1-DMHP (0·1 mg/kg i.v.). The immobility index of mice after injection of ^{3}H-Δ^1-THC (2 mg/kg) is also shown. Each point represents the mean of either six (20 min values) or four determinations (±S.E.M.).

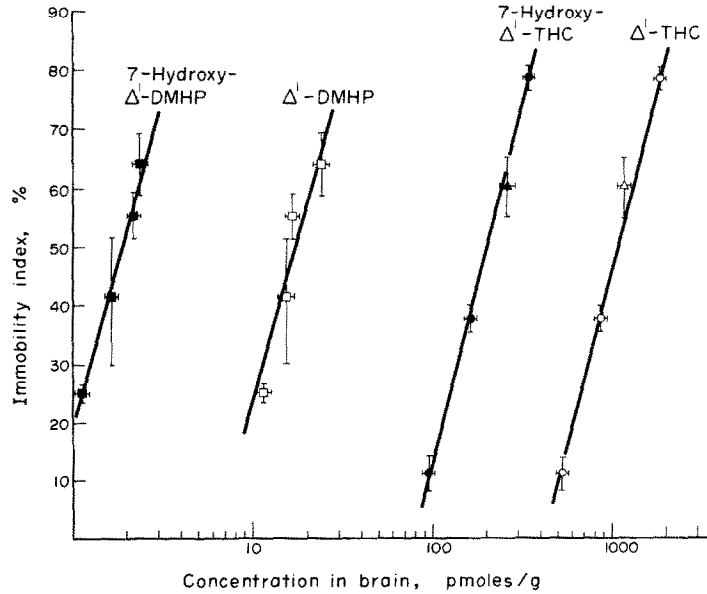

FIG. 4. Correlation of immobility index (±S.E.M.) measured at various times after injection of ^{3}H-Δ^1-DMHP (0·1 mg/kg), with whole brain concentrations of Δ^1-DMHP (□) and 7-hydroxy-Δ^1-DMHP (■). Similar results obtained previously[2] with Δ^1-THC are also shown (○ and ●), together with the single point for Δ^1-THC obtained from this work (△ and ▲). Points corresponding to the 3 lower levels of Δ^1-DMHP and its metabolite represent means for groups of four mice. Remaining points represent means for groups of six mice.

Figure 4 shows the strong linear correlation between immobility index and the log of the concentrations of Δ^1-DMHP and of 7-hydroxy-Δ^1-DMHP (correlation coefficients 0·95 and 0·99 respectively), the lines being approximately parallel not only to each other, but also to those obtained[2] for Δ^1-THC and 7-hydroxy-Δ^1-THC. The plots for Δ^1-THC and 7-hydroxy-Δ^1-THC were made using data obtained both in this work and in previously reported experiments.[2]

Lengthening the alkyl side-chain for cannabinoids appears to reduce the rate of penetration into the CNS. At all times up to 16 hr after injection the proportion of Δ^1-DMHP and its metabolites taken up into the brain was less than in the case of Δ^1-THC (Table 2). However the fraction of the injected dose which remained in the blood as the parent compound 20 min after injection was not significantly different between the Δ^1-DMHP and Δ^1-THC. Consequently the fraction of the injected dose of Δ^1-DMHP which reached the brain was lower than for Δ^1-THC (Fig. 5) and thus the molar potency ratio for Δ^1-DMHP and Δ^1-THC is underestimated by comparing intravenous doses: the relative brain concentrations of Δ^1-THC and Δ^1-DMHP at comparable levels of catalepsy being about 40:1 (Fig. 4) compared to the intravenous potency ratio of 12.

TABLE 2. MEAN CONCENTRATIONS (± S.E.M., GROUPS OF SIX MICE) OF CANNABINOIDS IN BLOOD AND BRAIN 20 MIN AFTER INTRAVENOUS INJECTION OF EITHER ^{3}H-Δ^1-DMHP (0·1 mg/kg) OR ^{3}H-Δ^1-THC (2·0 mg/kg)

Component	Concentration (pmoles/g ± S.E.M.)		Blood/brain ratio
	Brain	Blood	
Δ^1-DMHP	24·2 ± 2·2	89·0 ± 11·7	3·7
7-Hydroxy-Δ^1-DMHP	2·4 ± 0·4	26·8 ± 8·7	11·2
Polar metabolite	7·3 ± 1·6	72·2 ± 24·2	9·9
Non-extractable	10·8 ± 3·4	196·2 ± 56·8	18·2
Δ^1-THC	116·2 ± 76·3	2512·9*	2·2
7-Hydroxy-Δ^1-DMHP	261·6 ± 25·3	114·2*	0·4
Polar metabolite	154·1 ± 13·1	339·6*	2·2
Non-extractable	360·1 ± 122·3	325·9*	0·9

* Samples pooled from three mice.

Octanol–water partition coefficient. The octanol–water partition coefficient for Δ^1-DMHP was found to be 7500 ± 400, which compares with the value[10] of 6000 for Δ^1-THC. Thus although the increase in the length of the side-chain increases the lipid solubility, the magnitude of the effect is relatively small.

DISCUSSION

It has been shown that the Δ^1-dimethylheptyl analogue of Δ^1-THC is considerably more potent than Δ^1-THC in mice when assessed by the ring immobility test, whether the comparison is made on the basis of injected doses or of brain levels achieved. Loev *et al.*[6] have recently reported a potency ratio of 25 on the basis of the oral threshold doses required to produce behavioural changes in rats; this is in good agreement with the ratio of 12 found after intravenous administration to mice. On the other hand Mechoulam and Edery[1] found that following intravenous injection into rhesus monkeys Δ^1-DMHP was slightly less active than Δ^1-THC but that

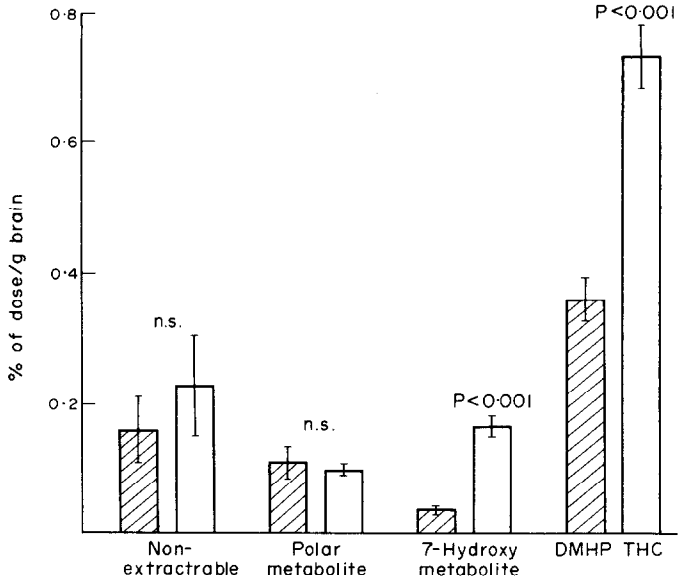

FIG. 5. Mean concentrations (expressed as the percentage of the injected dose per 1 g brain tissue ±S.E.M.) of the major components present in the brains of a group of six mice 20 min after injection of ^{3}H-Δ^1-DMHP (0·1 mg/kg; hatched) or ^{3}H-Δ^1-THC (2 mg/kg; open).

its effects were of very much longer duration. (24 hr as compared to 4·5 hr for comparable doses.) This is in complete contrast to the results obtained in mice, where the two compounds clearly differed in their potency but, at equipotent dose levels, produced effects of the same duration. Without further experimental data it is not profitable to speculate about this very marked difference in pharmacokinetic response between rhesus monkeys and mice.

Preliminary experiments with tritiated Δ^1-DMHP showed that the general pattern of metabolism was similar to that for Δ^1-THC, and a sample of the major *in vitro* metabolite was shown to have a mass spectrum consistent with 7-hydroxy-Δ^1-DMHP. Although this alone is not absolutely conclusive proof of the structure, it is sufficient to eliminate structures analogous to those found for other metabolites of Δ^1-THC, i.e. any side-chain hydroxylated compound, the 6α- and 6β-hydroxy compounds and the 6,7-dihydroxy compound. By analogy with other cannabinoids [(−)-Δ^1-THC,[16] (+)-Δ^1-THC,[3] cannabinol[17]] it seems unlikely that Δ^1-DMHP would have a completely different major metabolic pathway.

The results obtained with tritiated Δ^1-DMHP reveal that, although the general pattern of metabolism and distribution of Δ^1-DMHP is similar to that of Δ^1-THC, the differences are in an opposite sense to that required to give a simple explanation for the enhanced activity of the former. The fact that Δ^1-DMHP is rapidly converted in part to a metabolite that penetrates the CNS creates the same difficulty in interpretation as it does in the case of Δ^1-THC. However, as the ratio of metabolite to parent compound in the CNS is less in the case of Δ^1-DMHP it would be necessary to assume a very high potency ratio indeed between 7-hydroxy-Δ^1-DMHP and 7-hydroxy-Δ^1-THC for metabolic effects to account for the greater activity of Δ^1-DMHP. Δ^1-DMHP has a higher lipid solubility than Δ^1-THC and might be

expected to pass the blood–brain barrier more easily: it was found, however, that the fraction of the injected dose of Δ^1-DMHP which reached the brain was significantly less than for Δ^1-THC (Fig. 5), presumably due to stronger binding to some tissue binding site outside the CNS. This effect was consistent with the low fraction of material which could be extracted with ethyl acetate from the incubation mixture of Δ^1-DMHP with mouse liver. A similar explanation, though without any direct experimental evidence, has been advanced by Hansch *et al.*,[18] to account for an apparent optimum value of about 100 for the oil/water partition coefficient for a range of CNS depressants.

Studies on the pharmacokinetics of cannabis with human subjects must, perforce, be restricted to determination of plasma levels. The results of these and other[19] investigations using mice show that brain and plasma levels of cannabinoids often differ considerably, and that the plasma to brain concentration ratio depends upon the structure of the cannabinoid. Interpretations of the results of blood concentration determinations of cannabinoids must therefore be made with great caution. In the light of this and other work,[2,3] it must be concluded that the pattern of structure–action relationships found in the cannabinoid series assessed on the basis of administered doses is not significantly altered by differences in metabolism and tissue distribution and that discussions of the mode of action of cannabinoids at the molecular level must take account of the fact that the CNS site of action is asymmetric and capable of discriminating between closely related structures.

Acknowledgements—We are extremely grateful to Dr. R. T. Aplin for performing the g.l.c.-MS analyses, and to Miss Y. Beecher for performing the catalepsy assays. This work was supported by the Medical Research Council, and D. K. L. was the recipient of an M.R.C. Scholarship for training in research methods.

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