Biochemical Pharmacology, Vol. 22, pp. 1613–1621. Pergamon Press, 1973. Printed in Great Britain.

METABOLISM AND DISTRIBUTION OF CANNABINOIDS IN RATS AFTER DIFFERENT METHODS OF ADMINISTRATION*

EDITH G. LEIGHTY

Battelle, Columbus Laboratories, 505 King Avenue, Columbus, Ohio 43201, U.S.A.

(*Received* 17 *June* 1972; *accepted* 5 *January* 1973)

Abstract—Lungs of rats given ^{14}C-Δ^9-tetrahydrocannabinol (Δ^9-THC) by smoke inhalation showed a retention and metabolism of the cannabinoid, with further retention and metabolism by the liver. Only the liver showed retention and metabolism when ^{14}C-Δ^9-THC was given by i.v. injection. The brain showed a greater penetration or retention of unmetabolized cannabinoid after smoke inhalation than after i.v. injection. 11-Hydroxy-Δ^9-THC was observed soon after smoke inhalation of ^{14}C-Δ^9-THC in all of the organs and tissues except brain, and concentrations of a dihydroxy metabolite increased with time. A still unidentified metabolite was retained in the liver and spleen 15 days after an i.v. or chronic i.p. injection of ^{14}C-Δ^8-THC or ^{14}C-Δ^9-THC.

THE ACTIVE components of marihuana have been shown to be Δ^9-tetrahydrocannabinol (Δ^9-THC) and Δ^8-tetrahydrocannabinol (Δ^8-THC);[1,2] and since the Δ^9-isomer is the predominant compound, it is considered to be the principal active constituent.

Metabolic and distributional studies of Δ^9-THC, Δ^8-THC and other cannabinoids have previously been hampered by the lack of pure labeled compounds. Miras[3] measured the distribution of radioactivity in tissues of rats after intraperitoneal (i.p.) injections of a purified THC preparation from a plant grown in $^{14}CO_2$. With the synthesis of Δ^8-THC and Δ^9-THC, and the labeling of these compounds, first with ^{3}H[4,5] and later with ^{14}C,[6] additional metabolic studies became possible. The distribution and excretion of radioactive Δ^8-THC and Δ^9-THC have since been studied by oral, i.p., or intravenous (i.v.) administration in the rabbit,[7,8] the rat[9–11] and in humans.[12,13] These results showed that THC is rapidly extracted from the circulation by the liver and metabolized to more polar compounds, with excretion taking place primarily in the rabbit via the urine and in the rat and human via the feces. Very low levels of radioactivity were seen in the brain of the rat and rabbit after administration of THC by these methods. When ^{3}H-Δ^9-THC was given rats by smoke inhalation, the distribution pattern was very similar to that from the peritoneal route, except for initial retention by the lung.[14]

A metabolite of Δ^8-THC was found in our laboratories in the liver of rats injected *in vivo* with ^{14}C-Δ^8-THC[15] and could also be produced *in vitro* using the microsomal procedure of Dixon *et al.*[16] This metabolite was identified as 11-hydroxy-Δ^8-tetrahydrocannabinol (11-hydroxy-Δ^8-THC).[15] When Δ^9-THC was used as a precursor, a

* This investigation was supported by the Center for Narcotic Addiction and Drug Abuse Studies National Institute of Mental Health, under Contract No. PH-43-68-1338.

similar metabolite identified as 11-hydroxy-Δ^9-THC was also found by other investigators.[17,18] The metabolite of Δ^8-THC has also been isolated and identified in urine of rabbits.[19] Using a rat liver microsomal fraction, Wall *et al.*[18] found a further conversion of Δ^9-THC to 8, 11-dihydroxy-Δ^9-THC. Other workers have reported that cannabinol is also metabolized to 11-hydroxycannabinol.[20] The identification and distribution of the metabolites of Δ^8-THC and Δ^9-THC in the other organs and tissues of rats and rabbits have not been reported. However, a comparison was made by Christensen, *et al.*[21] of the relative proportions of Δ^9-THC to its total metabolites in specific organs of the mouse 10 min after intravenous or intracerebral administration of Δ^9-THC.

EXPERIMENTAL METHODS

The initial intravenous studies were performed using ^{14}C-Δ^8-THC.* After ^{14}C-Δ^9-THC became available, it was added to placebo marihuana for smoke inhalation studies and was also used for comparison with ^{14}C-Δ^8-THC in 15-day i.v. and i.p. cannabinoid retention studies.

Because of their insolubility in aqueous solvents, ^{14}C-Δ^8-THC and ^{14}C-Δ^9-THC were injected i.v. and i.p. as an ethanol–glycerol–saline emulsion made with Tween 80 into Wistar male rats (200–250 g) at a dose of 9 mg/kg (sp. act. 0·6–0·7 μCi/mg). In the smoke inhalation studies, four 200–250 g Wistar male rats at each time period were exposed to smoke from a reefer smoked in a positive pressure smoking machine. The reefer was made from 0·8 g placebo marihuana enriched with approximately 10 mg ^{14}C-Δ^9-THC (sp. act. 1·2 μCi/mg). The rats were contained in holders in an animal exposure chamber connected to the smoking machine and exposed to a 10:1 dilution with air of the smoke from the reefer. Recovery studies of the whole animal showed consistently that 1·0 $\pm$ 0·1% of the ^{14}C-Δ^9-THC in the reefer was transferred to each rat to give a dose of 0·6 $\pm$ 0·5 mg/kg. After i.v. injections of ^{14}C-Δ^9-THC, 75–85 per cent of the radioactivity was consistently recovered. Because of the limited supply of ^{14}C-Δ^9-THC, doses of higher concentrations were not investigated in the smoking studies.

At the designated interval after the termination of the smoking period (6 min), or after i.v. or i.p. injection, the rats were decapitated and exsanguinated. The blood was collected in a beaker containing EDTA and centrifuged to obtain plasma. The major organs were removed and the urine was collected from the bladder. The small intestines were rinsed with normal saline to obtain the fecal materials. Fat was taken from the perirenal depots and muscle tissue from the abdominal wall. In the initial experiments using ^{14}C-Δ^8-THC, a representative aliquot of the tissue or excretion product (about 200 mg) was homogenized and counted directly in Bray's solution by a Packard Tri-Carb liquid scintillation spectrometer, model 3375. In subsequent experiments, all samples were first homogenized in distilled water with a Polytron homogenizer, lyophilized, and an aliquot was then counted for radioactivity. The radioactivity in each aliquot was corrected for quenching from standard curves, and the total radioactivity of each sample determined. Quenching was not a serious problem, however, with any of the tissues except red cells. One experiment was

* ^{14}C-Δ^8-THC, ^{14}C-Δ^9-THC and placebo marihuana were supplied by the National Institute of Mental Health, Marihuana Research Program.

performed to compare any differences in total distribution of radioactivity found in lyophilized tissue with that obtained when only an aliquot of the sample was homogenized and counted. Less than 5 per cent difference was observed in the two methods. Lyophilization was preferred, however, since it facilitated the complete extraction of the radioactivity with methanol.

Usually three extractions with at least 15:1 (v/w) volume of methanol were sufficient to remove >95 per cent of the radioactivity. Portions of the extracted tissues were counted for radioactivity to ascertain that extraction was complete. The methanol extract of the tissue was dried under nitrogen and the dried residue redissolved in a known quantity of methanol. An aliquot of the extract was counted for radioactivity and another aliquot was spotted on Silica gel plates (Q-1, Quantum Industries) for separation of the radioactive cannabinoids. Solutions of Δ^8-THC, Δ^9-THC and 11-hydroxy-Δ^8-THC were also spotted on the same plate to give R_f reference standards. The plates were developed in heptane–benzene–methanol–ethyl acetate (55:15:20:10) and the standards and free cannabinoids of sufficient concentration in the extract were detected with a 0·5% solution of fast Blue B salt. Sections of the plate were scraped into counting vials containing Bray's solution and their radioactivity was determined. No loss of radioactivity was observed in the transfer of extract to thin-layer chromatography (TLC) plates.

In the 15-day experiments, 300-g rats were injected either i.v. at a single dose of 2·7 mg (9 mg/kg) or i.p. chronically for 5–10 days at a dose of 1 mg/day. The rats were placed in metabolic cages and the urine and feces collected and examined for radioactive cannabinoids at regular intervals. At the end of 15 days, the rats were killed and their major organs removed and analyzed for cannabinoids.

RESULTS AND DISCUSSION

Table 1 shows a comparison of the distribution of radioactivity in tissues and excretion products of rats given ^{14}C-Δ^8-THC by i.v. injection and ^{14}C-Δ^9-THC by smoke inhalation. Both routes of administration show a rapid uptake of radioactivity by the liver once the cannabinoids have entered the circulation. This was observed at 5 min by i.v. injection, but was not evident with smoke inhalation until approximately 30 min after administration. This lag in the smoke studies may be explained by the retention of cannabinoids in the lungs (>50 per cent up to 15 min), and gradual release into the blood. Very little radioactivity was present in the lungs at any time in the i.v. studies. This was not in agreement with the observations of Klausner and Dingell,[11] and Agurell *et al.*,[7] who found a high concentration of radioactivity in the lungs of rats and rabbits after an i.v. injection of radioactive Δ^9-THC.

A very low percentage of the total radioactivity was found in the brain. At 5 min, however, the percentage in the brain after smoke inhalation was twice that with i.v. injection. This becomes more noteworthy when it is observed that in all other organs, except lungs, the percentage was lower during the entire period. Smoke inhalation may thus be a more efficient method than i.v. injection for introducing cannabinoids into the brain of rats.

Increasing concentrations of radioactivity were found with time in the spleen in the i.v. studies. This may be related to the phagocytic behavior of the spleen toward large amounts of foreign substances. In the smoke studies, where the total concentration of

TABLE 1. PER CENT DISTRIBUTION OF RADIOACTIVITY IN RATS GIVEN ^{14}C-Δ^8-THC* INTRAVENOUSLY AND ^{14}C-Δ^9-THC† BY SMOKE INHALATION

	5 min‡		15 min‡		30 min‡		60 min‡		120 min‡	
	^{14}C-Δ^8-THC (i.v. injection)	^{14}C-Δ^9-THC (Smoke inhalation)	^{14}C-Δ^8-THC (i.v. injection)	^{14}C-Δ^9-THC (Smoke inhalation)	^{14}C-Δ^8-THC (i.v. injection)	^{14}C-Δ^9-THC (Smoke inhalation	^{14}C-Δ^8-THC (i.v. injection)	^{14}C-Δ^9-THC (Sooke inhalation)	^{14}C-Δ^8-THC (i.v. injection)	^{14}C-Δ^9-THC (Smoke inhalation)
Lungs	11·1 ± 0·7	56·7 ± 2·1	10·5 ± 0·7	57·1 ± 1·2	8·8 ± 1·1	35·0 ± 2·5	2·3 ± 0·2	31·8 ± 4·0	1·7 ± 0·6	12·6 ± 2·8
Spleen	10·7 ± 0·6	2·6 ± 0·1	11·5 ± 2·1	3·2 ± 0·2	13·4 ± 1·7	8·0 ± 0·8	14·2 ± 2·6	1·7 ± 0·5	14·9 ± 2·1	1·2 ± 0·5
Heart	17·3 ± 1·1	9·9 ± 0·9	11·9 ± 0·4	6·5 ± 0·3	8·3 ± 0·9	5·0 ± 0·5	2·3 ± 0·2	2·3 ± 0·2	1·7 ± 0·3	1·5 ± 0·3
Kidney	11·2 ± 0·6	5·2 ± 0·4	13·2 ± 0·8	5·9 ± 0·2	9·4 ± 0·8	4·3 ± 2·6	4·3 ± 0·8	2·9 ± 0·1	3·1 ± 0·5	3·8 ± 1·2
Liver	23·7 ± 2·3	7·8 ± 1·1	23·4 ± 1·8	6·9 ± 0·5	18·3 ± 1·8	12·3 ± 2·6	13·8 ± 3·4	6·0 ± 0·2	11·4 ± 1·1	3·4 ± 0·4
Brain	2·5 ± 0·1	5·0 ± 0·8	3·5 ± 0·1	3·8 ± 0·5	3·4 ± 0·3	3·2 ± 0·3	2·7 ± 0·6	2·0 ± 0·0	1·3 ± 0·1	1·7 ± 0·4
Fat	1·6 ± 0·4	3·0 ± 0·5	3·2 ± 0·6	4·0 ± 1·6	3·7 ± 0·5	2·5 ± 1·3	2·2 ± 0·3	1·8 ± 0·4	3·7 ± 0·4	3·7 ± 1·4
Muscle	2·4 ± 0·2	2·4 ± 0·2	2·7 ± 0·4	2·6 ± 0·6	3·2 ± 0·2	2·0 ± 0·2	1·5 ± 0·5	1·7 ± 0·1	1·1 ± 0·2	4·8 ± 1·2
Plasma	14·9 ± 0·6	1·6 ± 0·2	11·7 ± 1·3	3·4 ± 1·9	11·0 ± 1·5	0·4 ± 0·1	6·7 ± 1·2	0·3 ± 0·1	3·3 ± 0·1	0·2 ± 0·1
Red cells	1·7 ± 0·1	2·3 ± 0·1	3·0 ± 1·1	2·0 ± 0·3	2·6 ± 0·9	0·8 ± 0·2	1·5 ± 0·5	0·3 ± 0·1	0·6 ± 0·1	0·1 ± 0·1
Urine	0·1 ± 0·0	§	0·2 ± 0·0	0·9 ± 0·3	1·9 ± 0·8	13·9 ± 2·1	7·4 ± 0·6	6·9 ± 1·4	5·7 ± 1·4	7·1 ± 2·1
Feces	0·1 ± 0·0	§	0·7 ± 0·1	0·8 ± 0·2	11·9 ± 7·3	8·4 ± 3·3	34·8 ± 5·2	38·5 ± 2·6	48·3 ± 6·3	57·3 ± 6·1
Carcass	2·7 ± 0·2	3·6 ± 0·4	4·5 ± 0·6	2·9 ± 0·2	4·1 ± 0·6	4·2 ± 1·0	6·3 ± 0·9	3·8 ± 0·4	3·2 ± 0·2	2·6 ± 0·4

* Dose = 9 mg/kg.
† Dose = 0·6 ± 0·05 mg/kg.
‡ Results are average of four to five rats ± S.E.M. calculated from radioactivity recovered/g of sample.
§ No sample collected.

TABLE 2. THIN-LAYER CHROMATOGRAPHY OF CANNABINOIDS IN TISSUES AND EXCRETION PRODUCTS OF RATS GIVEN ^{14}C-Δ^9-THC BY SMOKE INHALATION

	5 min (TLC R_f*)			15 min (TLC R_f)			30 min (TLC R_f)			60 min (TLC R_f)			120 min (TLC R_f)		
	0·74	0·36	0·28	0·74	0·36	0·28	0·74	0·36	0·28	0·74	0·36	0·28	0·74	0·36	0·28
Lungs	29	37	29	15	58	27	4	68	28	11	16	66	7	24	66
Spleen	10	31	59	12	30	58	10	27	59	5	20	75	4	3	90
Heart	50	38	12	50	24	21	11	38	50	1	13	85		15	83
Kidney	52	42	6	50	20	26	29	8	58	24	6	69	10	7	80
Liver	26	55	18	7	45	47	7	42	48	3	12	81	4	10	84
Brain	89	8	2	77	18	5	70	21	9	29	13	54	24	15	55
Fat	31	62	5	1	76	21	8	66	26	7	23	70	5	6	88
Muscle	62	24	14	65	23	8	60	21	19	47	14	28	19	9	65
Plasma	1	95	2	1	41	56		45	55	4	1	90	5	7	88
Red cells		21	72		43	57	4	47	47	6	6	86		†	
Urine		‡		1	1	97		3	97	2	10	86	5	8	87
Feces		‡		4	8	85	6	4	87	3	3	93	6	4	89
Carcass	80	8	11	79	8	8	65	23	12	56	13	21	21	22	53

* TLC R_f: 0·74 corresponds with Δ^9-THC; 0·36 corresponds with 11-hydroxy-Δ^9-THC; 0·28 corresponds with 8,11-dihydroxy-Δ^9-THC reported by Wall[18]
Results expressed as percentage of total radioactivity on TLC plate.

† Total radioactivity too low for TLC.

‡No samples collected.

cannabinoids in the rat was much lower, decreased retention of radioactivity was observed after 30 min. In both studies, there was also very little retention of radioactivity by the other organs and tissues after 30 min. Excretion was primarily via the feces with both methods of administration.

A comparative study of ^{14}C-Δ^8-THC and ^{14}C-Δ^9-THC by the same method of i.v. injection showed very little difference in their ultimate retention, distribution, excretion or rate of metabolism.

Five min after smoke inhalation of ^{14}C-Δ^9-THC, unmetabolized Δ^9-THC comprised approximately 25 per cent of the cannabinoids found in the liver and lungs (Table 2). This was considerably lower than the 60 per cent unmetabolized Δ^8-THC observed in the liver 5 min after an i.v. injection of ^{14}C-Δ^8-THC (Table 3). Fifteen min after i.v. injection, unmetabolized ^{14}C-Δ^8-THC had only decreased to approximately 35 per cent of the cannabinoids in the liver, and this value remained constant up to 2 hr. In contrast to the relatively slow rate of decline of unmetabolized ^{14}C-Δ^8-THC in the liver after i.v. injection, very little unmetabolized ^{14}C-Δ^9-THC was seen in either the liver or lungs 15 min after smoke inhalation. The liver was the only organ examined for metabolites in the earlier ^{14}C-Δ^8-THC i.v. injection studies because of its enrichment in radioactive 11-hydroxy-Δ^8-THC, which we were interested in identifying.[15] The metabolites of ^{14}C-Δ^8-THC were also not differentiated from each other in the TLC system used at that time.

TABLE 3. PERCENTAGE OF UNMETABOLIZED Δ^8-THC IN CANNABINOIDS PRESENT IN LIVERS OF RATS GIVEN ^{14}C-Δ^8-THC INTRAVENOUSLY*

5 min	15 min	30 min	60 min	120 min
61·3 ± 3·6	37·4 ± 3·2	32·5 ± 1·8	33·0 ± 1·0	35·1 ± 6·9

* Results expressed as percentage of total radioactivity on TLC plate.

The increased percentage of metabolites observed in the liver of rats after smoke inhalation compared to i.v. injection, and the high percentage of metabolites present in the lungs, even at 5 min, suggested that the lung may also metabolize Δ^9-THC and Δ^8-THC before they reach the liver. Preliminary experiments in our laboratory have shown that metabolites of Δ^8-THC can be produced by a rat lung microsomal system *in vitro*. Subsequently, Nakazawa and Costa[22] have reported the metabolism of Δ^9-THC by lung homogenates of rats treated with methylcholanthrene. Since our results also showed the lungs to contain over 30 per cent of the cannabinoids observed in the rat 1 hr after smoke inhalation of ^{14}C-Δ^9-THC, the lungs may be of more importance than the liver for the study of the effects of smoking marihuana.

Δ^9-THC comprised nearly all of the cannabinoids seen in the brain up to 30 min after smoke inhalation of ^{14}C-Δ^9-THC. After 2 hr, it still constituted approximately 25 per cent of the cannabinoids present. Thus, Δ^9-THC either penetrates the brain easier than its metabolites, or is preferentially retained. This high percentage of Δ^9-THC relative to its metabolites in the early minutes after smoke inhalation, and its long retention in the brain, strongly support the hypothesis that it, rather than a metabolite, is the primary active component. When the behavioral effects of mari-

huana are known to be declining, we also see a decline in Δ^9-THC and an increase in its metabolites in the brain.

Although only a very low concentration of cannabinoids was present in any of the tissues and organs, except lungs, 2 hr after smoke inhalation of ^{14}C-Δ^9-THC, unmetabolized Δ^9-THC was still observed in the brain, muscle and carcass.

Very little free Δ^9-THC was observed in the blood at any time. The cannabinoids in the blood may be complexed or bound to a lipoprotein, as suggested by Wahlqvist *et al.*[23] since they moved very little on the TLC plate. Similarly, TLC immobility of the excretion products, especially the urine, suggests possible conjugation or formation of an additional metabolite(s) of higher polarity.

In the tissues and organs analyzed, the 11-hydroxy metabolite of Δ^9-THC was formed initially from the parent compound, with the dihydroxy metabolite then being formed in increasing concentrations with time. Thus, the metabolism of Δ^9-THC *in vivo* in the rat, up to 2 hr after smoke inhalation, appears to involve first the formation of a monohydroxy and then a dihydroxy metabolite.

Studies on the retention of cannabinoids in rats after i.v. injections of ^{14}C-Δ^8-THC or ^{14}C-Δ^9-THC showed that 50–60 per cent of the radioactivity from both of these compounds was excreted in the feces within 48–72 hr and 10–15 per cent in the urine within 24 hr. When they were injected i.p., approximately 30 per cent of the injection was recovered in the faeces and 8 per cent in the urine. This is in good agreement with the results found in rats by Miras,[3] when ^{14}C-Δ^9-THC was administered i.p., and also by Klausner and Dingell[11] and Agurell *et al.*,[10] when ^{14}C-Δ^9-THC and ^{3}H-Δ^9-THC were administered i.v. Certain organs and tissues of these rats examined 15 days after the initial injection showed a retention of radioactive cannabinoids (Table 4).

TABLE 4. RETENTION OF UNKNOWN METABOLITE IN ORGANS AND TISSUES OF RATS 15 DAYS AFTER INTRAVENOUS OR CHRONIC INTRAPERITONEAL INJECTIONS OF ^{14}C-Δ^8-THC OR ^{14}C-Δ^9-THC

	i.v. injection of 2·7 mg ^{14}C-Δ^8-THC (μg/g)	i.v. injection of 2·7 mg ^{14}C-Δ^9 THC (μg/g)	i.p. injection of 10 mg (1 mg/day) ^{14}C-Δ-^{8}-THC (μg/g)	i.p. injection of 5 mg (1 mg/day) ^{14}C-Δ^9-THC (μg/g)
Muscle	0·3 ± 0·1	0·3 ± 0·1	5·6 ± 2·0	1·6 ± 0·2
Fat	1·1 ± 0·2	3·7 ± 0·3	23·7 ± 4·8	7·8 ± 1·7
Brain	< 0·1 ± 0·0	< 0·1 ± 0·0	< 0·1 ± 0·0	< 0·1 ± 0·0
Kidney	0·3 ± 0.1	0·3 ± 0·1	2·1 ± 0·4	0·9 ± 0·2
Liver	2·7 ± 0·3	2·1 ± 0·3	18·9 ± 3·6	7·6 ± 2·1
Spleen	12·2 ± 2·3	9·3 ± 1·9	24·6 ± 5·8	8·4 ± 2·6

* Results are averages of five rats ± S.E.M.

The concentration of ^{14}C-Δ^9-THC retained in most cases was slightly less than that of ^{14}C-Δ^8-THC. This difference, however, may only be a result of a difference in actual injected dosages of Δ^8-THC or Δ^9-THC, since increased retention of cannabinoids was observed at the higher dose levels and later analysis showed that the Δ^9-THC used in these experiments was only of 75% purity.

With i.v. injection of both compounds, a high concentration of radioactive cannabinoids was observed in the spleen, with lesser amounts in the liver and fat. A small

amount of radioactivity was found in muscle and kidney. However, with both compounds and by both methods of injection, there was very little, if any, radioactivity retained in the brain.

A very similar retention pattern was observed with i.p. injection of larger amounts of both compounds. The spleen still showed the highest concentration, but the increase was lower relative to the other tissues. Increased retention of radioactivity was observed in the muscle and fat, especially at the higher concentration. No attempt was made to determine total recovery of radioactivity after i.p. injections, since we were primarily interested at that time in producing and attempting to identify an unknown metabolite retained in the liver and spleen. However, the increased concentration of radioactivity in muscle and fat, and decreased concentration in the excreta when compared to i.v. injections, indicate that there was incomplete absorption of THC.

Thin-layer chromatography of extracts of the livers and spleens removed after 15 days showed that an unidentified metabolite comprised at least 80 per cent of the radioactive cannabinoids retained by these organs. This unknown cannabinoid was present after both ^{14}C-Δ^8-THC or ^{14}C-Δ^9-THC injection, and after both methods of administration. It has a TLC R_f value (0·8) above that of Δ^8-THC or Δ^9-THC (0·74) in our developing system. It could also be found in the bone marrow, and was an increasing percentage of the cannabinoids found in feces, but not urine, after 5 days. Subsequent examination of this metabolite by gas–liquid chromatography–mass spectral analyses verified that this cannabinoid was not cannabinol, cannabidiol, Δ^8-THC, Δ^9-THC, or one of their hydroxy or carboxy metabolites. The identification of this metabolite is of importance because of its long retention, and possible interaction or interference with other drugs and metabolic processes, and is being pursued.

Acknowledgement—I wish to thank J. L. Walter and P. R. Webb for their fine technical assistance.

REFERENCES

1. Y. GAONI and R. MECHOULAM, *J. Am. chem. Soc.* **86,** 1646 (1964).
2. H. ISBELL, C. W. GORODETZSKY, D. JASINSKI, V. CLAUSEN, F. V. SPULAK, and F. KORTE, *Psychopharmacologia* **11,** 184 (1967).
3. C. J. MIRAS, in *Hashish, Its Chemistry and Pharmacology* (Eds. G. E. W. WOLSTENHOLME and J. KNIGHT), p. 37. Little, Brown, Boston (1965).
4. S. BURSTEIN and R. MECHOULAM, *J. Am. chem. Soc.* **90,** 2420 (1968).
5. J. L. G. NILSSON, I. M. NILSSON and S. AGURELL, *Acta chim. scand.* **23,** 2207 (1969).
6. M. L. TIMMONS, C. G. PITT and M. E. WALL, *Tetrahedron Lett.* No. 3129 (1969).
7. S. AGURELL, I. M. NILSSON, A. OHLSSON and F. SANDBERG, *Biochem. Pharmac.* **19,** 1333 (1970).
8. S. H. BURSTEIN, F. MENEZES, E. WILLIAMSON and R. MECHOULAM, *Nature, Lond.* **225,** 87 (1970).
9. R. F. TURK, J. E. MANNO, N. C. JAIN and R. B. FORNEY, *Pharmacologist* **11,** abstr. 286 (1969).
10. S. AGURELL, I. M. NILSSON, A. OHLSSON and F. SANDBERG, *Biochem. Pharmac.* **18,** 1195 (1969).
11. H. A. KLAUSNER and J. V. DINGELL, *Life Sci.* **10,** 49 (1971).
12. L. LEMBERGER, S. D. SILBERSTEIN, J. AXELROD and I. J. KOPIN, *Science, N.Y.* **170,** 1320 (1970).
13. L. LEMBERGER, N. R. TAMARKIN, J. AXELROD and I. J. KOPIN, *Science, N,Y.* **173,** 72 (1971).
14. B. T. HO, G. E. FRITCHIE, P. M. KRALIK, L. F. ENGLERT, W. M. MCISAAC and J. IDÄNPÄÄN-HEIKKILÄ, *J. Pharm. Pharmac.* **22,** 538 (1970).
15. R. L. FOLTZ, A. F. FENTIMAN, E. G. LEIGHTY, J. L. WALTER, H. R. DREWES, W. E. SCHWARTZ, T. F. PAGE and E. B. TRUITT, *Science, N.Y.* **168,** 844 (1970).
16. R. L. DIXON, L. G. HART, L. A. ROGERS and J. R. FOUTS, *J. Pharmac. exp, Ther.* **142,** 312 (1963).
17. I. M. NILSSON, S. AGURELL, J. L. G. NILSSON, A. OHLSSON, F. SANDBERG and M. WAHLQVIST, *Science, N.Y.* **168,** 1228 (1970).
18. M. E. WALL, D. R. BRINE, G. R. BRINE, C, G. PITT, R. I. FREUDENTHAL and H. D. CHRISTENSEN, *J. Am. chem. Soc.* **92,** 3466 (1970).

19. Z. Ben-Zvi, R. Mechoulam and S. Burstein, *J. Am. chem. Soc.* **92,** 3468 (1970).
20. M. Widman, I. M. Nilsson and J. L. G. Nilsson, *Life Sci.* **10,** 157 (1971).
21. 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, N.Y.* **172,** 165 (1971).
22. K. Nakazawa and E. Costa, *Nature, Lond.* **234,** 48 (1971).
23. M. Wahlqvist, I. M. Nilsson, F. Sandberg, S. Agurell and B. Granstrand, *Biochem. Pharmac.* **19,** 2579 (1970).