Biochemical Pharmacology, Vol. 35, No. 15, pp. 2553–2558, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

PROSTAGLANDINS AND CANNABIS—XVI

ANTAGONISM OF Δ^1-TETRAHYDROCANNABINOL ACTION BY ITS METABOLITES

SUMNER BURSTEIN,* SHEILA A. HUNTER, VAUGHAN LATHAM and LORI RENZULLI
Department of Biochemistry, University of Massachusetts Medical School, Worcester, MA 01605, U.S.A.

(*Received* 23 *September* 1985; *accepted* 23 *December* 1985)

Abstract—Prior exposure of cells *in vitro* to Δ^1-tetrahydrocannabinol-7-oic acid (Δ^1-THC-7-oic acid) reduced the degree of stimulation of prostaglandin synthesis incurred by subsequent treatment with Δ^1-THC. The site of action of this inhibitory effect seemed to be on cyclooxygenase and not at the earlier step involving the phospholipase-mediated release of arachidonic acid. Δ^1-THC-7-oic acid is a major metabolite of Δ^1-THC and has no psychoactivity in humans. Our findings raise the possibility, however, that it may influence the *in vivo* activities of Δ^1-THC by antagonizing its stimulatory action on cellular prostaglandin synthesis.

The discovery that the initial metabolites of Δ^1-tetrahydrocannabinol (THC) are active mood-altering agents has generated considerable interest in their role in the action of THC itself [1]. In particular, the monohydroxy derivatives have been studied in detail, and it is believed that they contribute to the overall effects of the drug, but that their presence is not required for the psychotropic action of cannabis [2].

Further metabolism of the hydroxy THCs leads ultimately to a series of carboxylic acid derivatives [3] which can be detected in the blood and other tissues some time after the THC levels have peaked [4, 5]. The most abundant member of this group is Δ^1-THC-7-oic acid and, this cannabinoid, when tested in humans [6] as well as in the rhesus monkey [7], showed no observable behavioral activity. Thus, little attention has been given to the possible pharmacodynamic properties of this metabolite or any of the other acid metabolites of THC.

For some time we have been studying the effects of THC and its metabolites on the transformations of arachidonic acid in a variety of *in vitro* systems with a view towards elucidating the mechanism of action of THC. These model systems display a surprising number of similarities to the properties of THC *in vivo*. For example, they show a similar structure–activity profile [8, 9], stereospecificity is exhibited†, and tolerance to the stimulatory effects of THC on prostaglandin (PG) synthesis can be observed [10].

In the above systems, THC-7-oic acid appears to be without any biological activity when assayed for stimulation of arachidonic acid metabolism. Since appreciable levels of this metabolite are generated during the course of action of THC *in vivo*, we were interested to see whether its presence would modify the action of THC in our *in vitro* model systems. The present report describes the extensive influence of the acid metabolite on the action of THC and suggests a possible mechanism for this effect.

MATERIALS AND METHODS

Chemicals. The cannabinoids were obtained from the National Institute on Drug Abuse, and their purity was monitored by reversed phase thin-layer chromatography. Whatman KP-18 plates were used, and the solvent system was 50% ethanol, 37.5% methanol, 12% water (pH 4.0). Bovine serum albumin (BSA), Fraction V, was purchased from the Sigma Chemical Co. (St. Louis, MO). [1-^{14}C]-Arachidonic acid (sp. act. 52.7 Ci/mmole) and [^{3}H]PGE_2 (sp. act. 165 Ci/mmole) were purchased from the New England Nuclear Corp. (Boston, MA). PGE antiserum was a gift from Dr. R. Skarnes, Worcester Foundation (Shrewsbury, MA). Its cross-reactivities with other prostaglandins were as follows: $PGF_{1\alpha}$, 4.0%; 6-keto-$PGF_{1\alpha}$, 1.0%; and PGD_2, <0.7%.

Fibroblasts. WI-38 human lung fibroblasts were seeded from stock originally obtained from Dr. L. Hayflick (University of Florida). The cells were grown to confluence ($2–3 \times 10^6$ cells/16 mm "miniwell") in Eagle's Minimum Essential Medium (with Earle's salts) (MEM) containing 10% fetal calf serum.

Preparation of macrophages. The cells were obtained by peritoneal lavage with MEM from 90-day-old mice (Charles River CD-1). Ten mice were used for each experiment yielding approximately 50×10^6 cells in 50 ml MEM to which penicillin–streptomycin (100 units/ml–100 μg/ml) was added. The cell suspension was divided, added to twenty-four "miniwells" and allowed to attach overnight in

* Author to whom all correspondence should be sent.

† S. Burstein, S. A. Hunter, V. Latham, L. Renzulli, and R. Mechoulam, unpublished observations.

an incubator at 37° in an atmosphere of 95% O_2:5% CO_2.

Arachidonate labeling and release. The cells were incubated with [1-^{14}C]arachidonic acid (75,000 dpm) in 0.5 ml of serum-free MEM for 60 min. The cells were then washed free of unreacted arachidonic acid with MEM and immediately used for the release studies. Under these conditions, 50–70% of the incorporated radioactivity is present as phospholipids [11].

The labeled cells were incubated at 37° for 30 min with Δ^1-THC-7-oic acid delivered in 10 μl ethanol to the medium (0.5 ml MEM + 0.1% BSA). The medium was then removed and centrifuged for 5 min at 3000 *g* to remove any suspended cells or membrane fragments. Duplicate aliquots of the medium were then assayed for carbon-14 by liquid scintillation counting. The results are expressed as the percentage of incorporated radioactivity released to the medium. Cell survival was monitored by measuring cellular DNA and ranged from 80 to 100% of control.

Prostaglandin synthesis measurements. Cells were washed free of serum with MEM and incubated for 30 min with 0.5 ml MEM containing the cannabinoid or its vehicle (10 μl ethanol). The media were then collected and centrifuged for 5 min at 3000 *g* to remove cells and membrane fragments. Duplicate aliquots were then assayed for PGE_2 using the antiserum described above and the radioimmunoassay procedure described previously [12]. The results are expressed as nanograms of PGE_2 per milliliter of culture medium. This antiserum does not distinguish between PGE_1 and PGE_2; however, it has been shown that these cells produce primarily PGE_2 [13].

Preparation of brain subcellular fractions. Briefly, brains from cervically dislocated CD-1 mice were rapidly excised and placed into ice-cold 0.32 M sucrose:0.05 M Tris–HCl buffer (pH 7.4):1 mM EDTA:10 mM $MgCl_2$ (STEM buffer). Each brain was homogenized in 6 ml STEM buffer with a Teflon pestle and homogenizer (radial clearance of 0.004 to 0.006 inch) for twelve strokes. After standing on ice for 90 sec, the preparation was homogenized again for eight strokes and centrifuged at 900 *g* for 10 min to yield a cellular debris and nuclear pellet. The supernatant fraction was centrifuged at 20,000 *g* for 15 min to obtain a pellet comprised of mitochondria, synaptosomes and myelin. Microsomes were obtained from the supernatant fraction by centrifugation at 105,000 *g* for 60 min. The pellet was suspended in 0.32 M sucrose:0.05 M Tris–HCl buffer (pH 7.4) to give a protein concentration of 0.78 mg/ml (determined by the Lowry procedure).

Preparation of fibroblast microsomes. WI-38 fibroblasts were grown to confluence (12×10^8 cells) in a roller bottle in MEM with 10% fetal calf serum, scraped from the flask, and homogenized in 0.32 M sucrose: 0.05 M Tris–HCl buffer (pH 7.4). The suspension was centrifuged at 10,000 *g* for 15 min; the supernatant fraction was then centrifuged for 60 min at 105,000 *g*. The pellet was then suspended in the above buffer to give a protein concentration of 0.19 mg/ml.

Assay for PG cyclooxygenase activity. A purified enzyme preparation from ram seminal vesicles (RSV) was obtained from Oxford Biomedical Research, Inc. (Oxford, MI). Each incubation mixture contained 0.95 ml Tris buffer (0.1 M, pH 7.4), reduced glutathione (5 μM), epinephrine (0.5 mM) and 10 units of enzyme. After 1 min, hematin was added to give a concentration of 0.1 μM. The inhibitor to be tested or vehicle (10 μl ethanol) was then added, and this mixture was incubated for 1 min. The reaction was initiated by adding the substrate [^{14}C]arachidonic acid (25,000 dpm) in ethanol (10 μl), and the incubation was allowed to proceed for 10 min in air at 37°. The mixture was then acidified with 1 N sulfuric acid (0.5 ml) and extracted with ethyl acetate (5 ml). The organic phase was dried and evaporated with a stream of nitrogen, and the residue was chromatographed on a silica gel plate using chloroform–methanol–acetic acid (90:6:6) as the eluent. The zones corresponding to added standards were removed, and the carbon-14 content was measured by liquid scintillation counting.

RESULTS

A potent stimulatory action of Δ^1-THC on PGE_2 synthesis in WI-38 human lung fibroblasts was observed (Fig. 1), in agreement with our earlier findings [8, 10, 12]. The addition of 8 μM and 16 μM Δ^1-THC-7-oic acid to the culture media prior to Δ^1-THC exposure resulted in a dramatic lowering of the stimulatory effect of Δ^1-THC on PGE_2 synthesis (Fig. 1). The limited amount of data did not permit

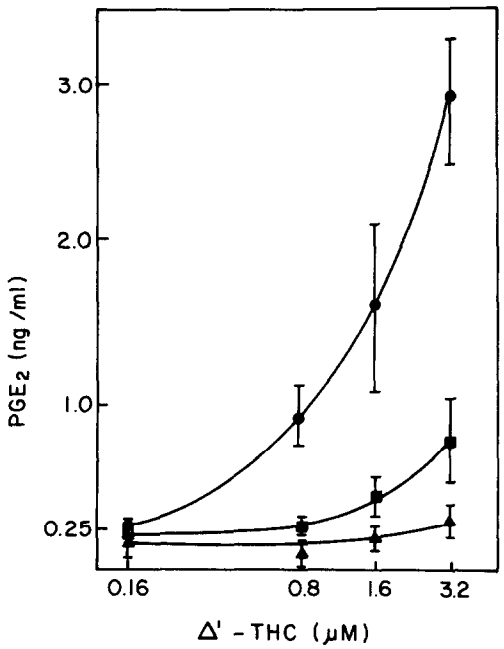

Fig. 1. Inhibition of PGE_2 synthesis by Δ^1-THC-7-oic in THC-stimulated WI-38 human lung fibroblasts. Cells were exposed to vehicle (95% ethanol; ●) or 7-oic acid (■, 8 μM; ▲, 16 μM) for 30 min and immediately challenged with Δ^1-THC for another 30 min. PGE_2 levels were measured by RIA as described in Materials and Methods. Values shown are the mean ± S.D. of duplicate determinations done on six individual monolayers.

Table 1. Interactions of Δ^1-THC with its metabolites on PGE_2 synthesis in fibroblasts*

Metabolite	Ratio of PGE_2 levels† (treated/control) Δ^1-THC concentration (μM) 0.32	0.80	1.6	3.2
6α-OH-Δ^1-THC (3.2 μM)	0.93	0.93	1.76	0.99
6α-OH-Δ^1-THC (8.0 μM)	1.15	0.93	1.26	0.89
1″-OH-Δ^1-THC (3.2 μM)	1.31	1.44	0.93	1.07
1″-OH-Δ^1-THC (8,0 μM)	2.16	1.89	1.46	1.45
1″-oic Acid (16 μM)	0.96	0.93	1.43	1.56
2″-oic Acid (16 μM)	0.83	0.49	0.51	0.74
3″-oic Acid (16 μM)	1.43	1.14	1.20	0.87
7-oic Acid (8 μM)		0.28	0.28	0.27
7-oic Acid (16 μM)		<0.15	0.13	0.11

* The metabolite was added to the cells 30 min prior to the addition of Δ^1-THC at the indicated concentrations.

† Controls consisted of cells to which vehicle (10 μl ethanol) had been added in place of a metabolite. PGE_2 was measured by RIA as described in Materials and Methods. Duplicate determinations were done on three individual monolayers.

an accurate kinetic analysis; however, a Cornish–Bowden–Eisenthal plot suggested that the inhibition was non-competitive.*

Other metabolites of Δ^1-THC were also studied for antagonist activity in the fibroblast-PGE_2 model used above. Two monohydroxy derivatives and three metabolites in which the side chain of THC has been oxidized to carboxylic acid functions of various lengths were studied at doses ranging from 3.2 to 16 μM (Table 1). The only metabolite showing inhibition approaching that of the 7-oic acid was the 2″-oic acid. Thus, there would seem to be somewhat specific structural requirements for the observed antagonism.

The inhibitory action of the 7-oic acid was not observed when the release of free arachidonic acid was measured (Table 2). The model for this experiment was a monolayer of mouse peritoneal macrophages in which the phospholipid pools had been labeled with [^{14}C]arachidonic acid as previously described by us [9]. The data shown in Table 2 indicate that pretreatment of the cells with 7-oic acid did not reduce the THC-induced stimulation of hydrolysis when compared with vehicle-pretreated macrophages. In fact, a small agonist effect was seen when a dose of 1.6 μM THC was used.

A study similar to that described above was carried out, and the media were analyzed by thin-layer chromatography to determine the composition of products originating from arachidonic acid. The most significant changes due to 7-oic acid pretreatment were a decrease in PGE_2 and increases in zones where the HETES would be expected (Table 3). The amount of carbon-14 in zone 5 also decreased; however, the identity of this material is not known. Thus, it seems that the effect of the 7-oic acid is to shift precursor arachidonate from the cyclooxygenase pathway into the lipoxygenase pathways.

Table 4 shows the inhibitory effects of the 7-oic acid on the cyclooxygenase activities of broken cell and microsomal preparations from WI-38 fibroblasts. The two major cyclooxygenase products in this cell type are PGE_2 and TxA_2 [13]. We found dose-related decreases in both of these when [^{14}C]arachidonate was the precursor and the products analyzed by TLC. A comparison with indomethacin showed that this inhibitor of PG synthesis was equipotent at about one-tenth the dose of THC-7-oic acid.

The effects of the 7-oic acid on mouse brain cyclooxygenase activity were more complex, as shown by the data in Table 5. Inhibition was observed in the production of both $PGF_{2\alpha}$ and TxB_2; however, the formation of PGE_2 was stimulated at higher doses. In contrast, the synthesis of both $PGF_{2\alpha}$ and PGE_2 by a brain microsomal preparation was inhibited by the 7-oic acid; TxB_2 was not measured in this experiment.

To minimize any effects of nonenzymic impurities in the cyclooxygenase preparations, we obtained a commercially available sample of electrophoretically purified material. This cyclooxygenase had been prepared from ram seminal vesicles and is claimed to be more than 85% pure. Δ^1-THC-7-oic acid was effective in reducing PGE_2 and TxB_2 levels; however, no inhibition of $PGF_{2\alpha}$ synthesis was found (Table 6).

DISCUSSION

The data presented in this report clearly indicate that Δ^1-THC-7-oic acid, a major metabolite of Δ^1-THC, can antagonize the *in vitro* action of the parent

* A. Carruthers, personal communication.

Table 2. Δ^1-THC elevated phospholipase activity in macrophages pretreated with Δ^1-THC-7-oic acid*

Δ^1-THC (μM)	[^{14}C]Arachidonate release (%) Vehicle pretreated	Δ^1-THC-7-oic acid pretreated	P
8.0	7.94 ± 0.64	7.95 ± 1.19	>0.05
3.2	6.86 ± 0.61	6.27 ± 0.66	>0.05
1.6	3.84 ± 0.66	4.62 ± 0.11	>0.05

* Cells were obtained as described in Materials and Methods. Each monolayer was exposed to either vehicle (10 μl ethanol) or Δ^1-THC-7-oic acid for 30 min followed by a 30-min exposure to Δ^1-THC. Values are means ± S.D., N = 3. Statistical significance was determined by Student's *t*-test.

Table 3. Product composition in media from [^{14}C]arachidonate-labeled macrophages*

TLC zone†	% ^{14}-Carbon content (dpm)	
	Control	Δ^1-THC-7-oic acid treated
3 ($PGF_{2\alpha}$)	14.3 (371)	13.3 (470)
4 (PGE_2)	22.4 (580)	16.8 (594)
5 ?	42.0 (1086)	31.3 (1106)
6 (HETES)	13.8 (357)	26.6 (938)
7 (HETES)	3.6 (93)	4.8 (168)
8 (Arachidonate)	3.9 (101)	7.2 (256)

* Cells were prepared and treated as in Table 2 and labeled as described in Materials and Methods.
† See Ref. 9 for TLC and extraction conditions.

Table 4. Inhibition of fibroblast cyclooxygenase activity by Δ^1-THC-7-oic acid

Conditions	Change in ^{14}C-labeled products* (%)		
	PGE_2	TxB_2	Arachidonate
Broken cells†			
Indomethacin (0.3 μM)	−21	−30	+3.0
Δ^1-THC-7-oic-acid (3.2 μM)	−35	−30	+3.8
Δ^1-THC-7-oic acid (8 μM)	−44	−40	+4.6
Δ^1-THC-7-oic-acid (16 μM)	−54	−55	+6.1
Microsomes†			
Δ^1-THC-7-oic-acid (16 μM)	−16	ND§	+2.5

* See Ref. 8 for TLC and extraction conditions.
† Prepared and assayed as described in Materials and Methods.
§ Not determined.

Table 5. Effect of Δ^1-THC-7-oic acid on mouse brain cyclooxygenase transformations of [^{14}C]arachidonic acid*

Conditions	Change in ^{14}C-labeled products† (%)			
	$PGF_{2\alpha}$	TxB_2	PGE_2	Arachidonate
20,000 *g* supernatant fraction				
Δ^1-THC-7-oic acid (3.2 μM)	−29	−17	−17	+1.7
Δ^1-THC-7-oic acid (16 μM)	−38‡	−34	0	+1.3
Δ^1-THC-7-oic acid (32 μM)	−47‡	−34	+17	+0.4
Indomethacin (16 μM)	−47‡	−17	−50‡	+1.0
Microsomes				
Δ^1-THC-7-oic acid (16 μM)	−40‡	ND§	−25.5	+0.83‡

* See Materials and Methods for details. TLC conditions are described in Ref. 8.
† Values are the percent change compared with the vehicle control (10 μl ethanol).
‡ Significantly different ($P < 0.05$) from control values using Student's *t*-test. N = 3.
§ Not determined.

Table 6. Effect of Δ^1-THC-7-oic acid on purified cyclooxygenase transformations of [^{14}C]arachidonic acid*

Conditions	Change in ^{14}C-labeled products† (%)			
	$PGF_{2\alpha}$	TxB_2	PGE_2	Arachidonate
Δ^1-THC-7-oic acid (3.2 μM)	+12.0‡	−26.1‡	−11.6‡	+14.5‡
Δ^1-THC-7-oic acid (8.0 μM)	+0.59	−27.6‡	−11.6‡	+65.6
Δ^1-THC-7-oic acid (16 μM)	+4.07	−43.4‡	−17.7‡	+87.5‡
Indomethacin (16 μM)	−73.3‡	−68.9‡	−69.4‡	+60.0‡

* See Materials and Methods for details. TLC conditions are described in Ref. 8.
† Values are the percent change compared with the vehicle control (10 μl ethanol).
‡ Significantly different ($P < 0.05$) from control values using Student's *t*-test. N = 3.

substance. The system in which we have observed this effect involves the exposure of cells in culture to cannabinoids and measurement of the changes brought about in the metabolism of arachidonic acid [8, 11, 12]. In this model, THC shows a clear stimulatory action on the release of arachidonic acid from its phospholipid storage sites and the subsequent transformation of the free arachidonate into either cyclooxygenase or lipoxygenase products. This *in vitro* action may be relevant to some of the *in vivo* activities of THC including its psychotropic effects [14].

If our *in vitro* model does, in fact, represent *in vivo* events, then our present findings on the antagonism of the 7-oic acid suggest the possibility of an interesting pharmacokinetic–pharmocodynamic relationship. It has been well documented that the body levels of Δ^1-THC-7-oic acid are on the rise as the effects of the drug are beginning to wane [4, 5]. Thus, it may be that the tissue level of 7-oic acid is an important factor in determining the intensity of THC action at a particular time point during the exposure of the subject to the drug.

We have also presented data which partially elucidate the mechanism of this antagonism by Δ^1-THC-7-oic acid. Other metabolites of Δ^1-THC were tested *in vitro*, and only one showed significant activity in blocking the stimulatory action of THC (Table 1). This suggests that the effect has fairly strict inhibitor structure requirements and that a specific site in the cell may be involved.

Δ^1-THC acts primarily on the phospholipases which release precursor arachidonic acid for prostaglandin synthesis in the cell [8, 9, 11]. In broken cell preparations such as microsomes, inhibition of PG synthesis by cannabinoids can be seen [15]; however, in intact cells under ordinary conditions only stimulation is observed. We examined the effect of the 7-oic acid on THC-stimulated release of arachidonate (Table 2); no inhibition by the metabolite was observed, demonstrating that it must exert its effect at a later stage in the biosynthesis of the prostaglandins. Chromatographic analysis of the release experiment (Table 2) suggested that cyclooxygenase was the site of inhibition by the 7-oic acid since conversions to PGE_2 and $PGF_{2\alpha}$ were diminished while lipoxygenase transformations were increased.

Similar experiments were carried out on several different cyclooxygenase preparations giving direct evidence for this enzyme as the site of interaction with the 7-oic acid. The transformation of ^{14}C-labeled arachidonate to PGE_2 and TxA_2 in WI-38 lung fibroblast fractions was inhibited by the 7-oic acid (Table 4). Thus, our results mentioned above on the whole cells could be explained by a specific action at this biosynthetic step. We also carried out a similar study with mouse brain fractions (Table 5). In that system, $PGF_{2\alpha}$ and thromboxane levels were reduced at all doses (3.2 to 32 μM); however, a biphasic effect was seen when PGE_2 was measured. Such complex responses to drug action on cyclooxygenase have been reported for both cannabinoids [16] and other drugs [17]. Even when tested with a relatively pure preparation of cyclooxygenase, this dual effect was observed. Table 6 shows that, in a purified preparation from ram seminal vesicle, $PGF_{2\alpha}$ was elevated, whereas TxB_2 and PGE_2 were depressed at the same doses of Δ^1-THC-7-oic acid. There is no obvious explanation for this behavior at this time; however, it seems that the product composition is sensitive to the drug dose, the tissue source of the enzyme, etc. It should be noted that, in every case which we tested, arachidonate levels were increased, indicating that the net effect is an inhibition of the transformations beyond the release of free arachidonate.

From the chemical structure point of view it is not too surprising that Δ^1-THC-7-oic acid is a cyclooxygenase inhibitor. The structural requirements for the nonsteroidal anti-inflammatory agents (NSAID) have been studied in some detail because of their great importance as therapeutic agents [18]. The structure of the 7-oic acid would seem to fit the requirements set forth for an NSAID, and it might be interesting to do a detailed comparison of steric and electron density factors of this cannabinoid with other NSAIDs.

In conclusion, several points can be made about Δ^1-THC-7-oic acid and possibly other acid metabolites of THC. First, it may have an important role in defining the length and intensity of action of THC, particularly the cardiovascular effects and possibly also its psychoactivity. Second, the metabolizing capability of the subject exposed to THC may have an immediate and direct effect on its pharmacodynamic properties, in addition to the effects on clearance of the drug. Finally, this hitherto unimportant cannabinoid may serve as a model for a new class of NSAIDs.

Acknowledgements—We wish to thank John Cruz for growing the fibroblasts used in this study and Dr. Arthur Blume of the Roche Institute for stimulating discussions. The research was supported by Grants DA02043 and DA02052 from the National Institute on Drug Abuse. One of us (S. B.) is also the recipient of a Research Scientist Award from NIDA.

REFERENCES

1. W. D. M. Paton, *A. Rev. Pharmac.* **15**, 195 (1975).
2. D. J. Harvey and W. D. M. Paton, *Rev. biochem. Toxic.* **6**, 250 (1984).
3. S. Burstein, J. Rosenfeld and T. Wittstruck, *Science* **176**, 422 (1972).
4. M. E. Wall and M. Perez-Reyes, *J. clin. Pharmac.* **21**, 178S (1981).
5. R. L. Foltz and B. J. Hidy, in *Analysis of Cannabinoids* (Ed. R. L. Hawks), pp. 99–118. NIDA Res. Monograph 42 (1982).
6. M. Perez-Reyes, in *Pharmacokinetics and Pharmacodynamics of Psychoactive Drugs* (Eds. G. Barnett and N. Chiang), pp. 287–310. Biomedical Press (1985).
7. R. Mechoulam and H. Edery, in *Marijuana* (Ed. R. Mechoulam), Chap. 2. Academic Press, New York (1973).
8. S. Burstein, S. A. Hunter and K. Ozman, *Molec. Pharmac.* **23**, 121 (1983).
9. S. Burstein, S. A. Hunter, K. Ozman and L. Renzulli, *Biochem. Pharmac.* **33**, 2653 (1984).
10. S. Burstein, S. A. Hunter and L. Renzulli, *J. Pharmac. exp. Ther.* **235**, 87 (1985).
11. S. Burstein and S. A. Hunter, *J. clin. Pharmac.* **21**, 240S (1981).

12. S. Burstein, S. A. Hunter, C. Sedor and S. Shulman, *Biochem. Pharmac.* **31**, 2361 (1982).
13. R. W. Bryant, S. J. Feinmonk, H. J. Makkeja and J. M. Bailey, *J. biol. Chem.* **253**, 8134 (1978).
14. S. Burstein, S. A. Hunter, K. Ozman and L. Renzulli, in *Marihuana '84: Proceedings of the Oxford Symposium on Cannabis* (Ed. D. J. Harvey), pp. 399–406. IRL Press, Oxford (1984).
15. S. Burstein, E. Levin and C. Varanelli, *Biochem. Pharmac.* **22**, 2905 (1973).
16. C. O. Haavik, K. Crowshaw, F. G. Collins and H. H. Hardman, in *Proceedings of the Symposium on Temperature Regulation and Drug Action, Paris 1974* (Eds. P. Lomax and E. Schönbaum), pp. 310–8. Karger, Basel (1975).
17. D. C. Atkinson and H. O. J. Collier, *Adv. Pharmac. Chemother.* **17**, 248 (1980).
18. W. E. M. Lands and A. M. Hanel, in *Prostaglandins and Related Substances* (Eds. C. Pace-Asciak and E. Granstrom), Chap. 6. Elsevier, Amsterdam (1983).