



# The Cannabinoid Acids: Nonpsychoactive Derivatives with Therapeutic Potential

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**ABSTRACT.** The discovery of carboxylic acid metabolites of the cannabinoids (CBs) dates back more than three decades. Their lack of psychotropic activity was noted early on, and this resulted in a total absence of further research on their possible role in the actions of the CBs. More recent studies have revealed that the acids possess both analgesic and anti-inflammatory properties and may contribute to the actions of the parent drug. A synthetic analog showed similar actions at considerably lower doses. In this review, a brief survey of the extensive literature on metabolism of  $\Delta^9$ -tetrahydrocannabinol to the acids is presented, while more emphasis is given to the recent findings on the biological actions of this class of CBs. A possible mechanism involving effects on eicosanoids for some of these actions is also suggested. Finally, an analogy with a putative metabolite of anandamide, an endogenous CB, is discussed. PHARMACOL. THER. 82(1):87–96, 1999. © 1999 Elsevier Science Inc. All rights reserved.

**KEY WORDS.** Cannabinoid, metabolites, analgesia, anti-inflammatory activity, anandamide.

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**ABBREVIATIONS.** CB, cannabinoid; COX, cyclooxygenase; DMH, dimethylheptyl; HPETE, hydroperoxy-eicosatetraenoic acid; LOX, lipoxygenase; NSAID, nonsteroidal anti-inflammatory drug; PAF, platelet-activating factor; PG, prostaglandin; SB-1, arachidonyl glyceryl amide; SB-2, arachidonyl  $\gamma$ -aminobutyryl amide; SB-3, *p*-arachidonylamido phenol; THC, tetrahydrocannabinol.

## 1. INTRODUCTION

The psychotropic principle of *Cannabis* is tetrahydrocannabinol (THC), a highly lipophilic molecule whose structure is shown in Fig. 1. The recent discovery of two G-protein-coupled transmembrane cannabinoid (CB) receptors, CB1 and CB2, has generated a new wave of interest in this class of drugs (Pertwee, 1997). Hope has been renewed that the long-sought goal of separation of psychoactivity from the medicinal properties of *Cannabis* can now be achieved through rational drug design. While this approach has merit and has already led to several interesting candidate molecules, a second strategy has also resulted in some promising leads. This latter approach is based on the properties of the acid metabolites of  $\Delta^9$ -THC, which show little or no psychoactivity (Burstein *et al.*, 1988; Watanabe *et al.*, 1980;

Perez-Reyes, 1985) and do not bind to CB1 or CB2 (Rhee *et al.*, 1997).

## 2. METABOLITES OF TETRAHYDROCANNABINOL

### 2.1. Pathways of Metabolism

$\Delta^9$ -THC is rapidly metabolized in the body to a number of oxygenated products (for reviews, see Agurell *et al.*, 1986; Burstein, 1985; Harvey and Paton, 1984); however, the most important route in most species involves oxidation of the allylic methyl group, and is outlined in Fig. 2 (Burstein *et al.*, 1972). Oxidation may occur elsewhere in the molecule, notably at various positions on the side chain, leading to polyfunctional metabolites (Fig. 3). The first hint that there were acid metabolites of  $\Delta^9$ -THC was reported by

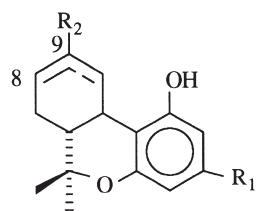


FIGURE 1. CB structures. See Fig. 3 for the numbering system. DMH-THC-11-oic acid is code named CT3.

	R <sub>1</sub>	R <sub>2</sub>
Δ <sup>8</sup> -THC (solid line)	n - pentyl	methyl
Δ <sup>9</sup> -THC (broken line)	n - pentyl	methyl
THC - 11 - oic acid	n - pentyl	carboxyl
DMH - THC - 11 - oic acid (CT3)	1',1'- dimethylheptyl	carboxyl

Aguirell *et al.* (1970), who found an acidic fraction in the urine of rabbits given radiolabeled Δ<sup>9</sup>-THC. Subsequent studies by several groups demonstrated the importance of this route of metabolism for Δ<sup>9</sup>-THC and the other major CBs in *Cannabis* in most species, including humans (Wall and Perez-Reyes, 1981; Widman *et al.*, 1985). Due to the prevalence and persistence of the acid metabolites in human tissues, these metabolites have become the basis for most of the drug abuse screening methods for the detection of *Cannabis* usage. Thus, habitual *Cannabis* users are chronically exposed to relatively high plasma levels (65–70 ng/mL) of Δ<sup>9</sup>-THC-11-oic acid (Wall and Perez-Reyes, 1981). These authors also reported that direct administration of Δ<sup>9</sup>-THC-11-oic acid to human subjects produced no psychotropic responses and resulted in very little further metabolism of the acid other than the formation of glucuronide conjugates.

## 2.2. Biological Activities of the Metabolites

A remarkable change in the biological activities of THC occurs during the course of its metabolism (Fig. 2). While monohydroxy Δ<sup>9</sup>-THC and its aldehyde product (not shown) have pharmacological profiles similar to Δ<sup>9</sup>-THC, the terminal carboxy metabolite has no psychotropic effect in humans (Perez-Reyes, 1985) and does not produce the behavioral responses typical of Δ<sup>9</sup>-THC in laboratory animals (Burstein *et al.*, 1988; Watanabe *et al.*, 1980). In fact, Δ<sup>8</sup>-THC-11-oic acid has been shown to attenuate the cataleptic effect of Δ<sup>9</sup>-THC in mice (Burstein *et al.*, 1987) by an undetermined mechanism.

Inhibition by Δ<sup>9</sup>-THC-11-oic acid of one of the *in vitro* effects of Δ<sup>9</sup>-THC has also been reported some time ago (Burstein *et al.*, 1986a). It was shown that the acid was able to reduce Δ<sup>9</sup>-THC-induced synthesis of immunoreactive prostaglandin (PG)E<sup>1</sup> in WI-38 human lung fibroblasts, probably by inhibiting the action of cyclooxygenase (COX). Inhibition of PG synthesis is a typical property of the non-

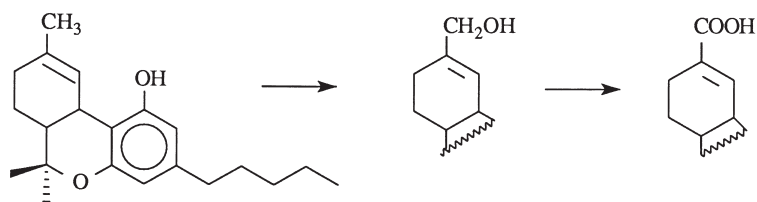
steroidal anti-inflammatory drugs (NSAIDs), and this observation (Burstein *et al.*, 1986a) suggested that Δ<sup>9</sup>-THC-11-oic acid may exhibit similar effects in experimental models of pain and inflammation. This hypothesis was tested in several such models, and it was observed that the acid behaved as an NSAID in the mouse hot-plate test (Burstein *et al.*, 1988; Doyle *et al.*, 1990), platelet-activating factor (PAF) and arachidonate-induced paw edema tests (Burstein *et al.*, 1989), the paraphenylquinone writhing assay (Doyle *et al.*, 1990), and PAF-induced mortality (Burstein *et al.*, 1989). These findings raised the possibility that the analgesic and anti-inflammatory properties of Δ<sup>9</sup>-THC may be due, at least in part, to its acid metabolites.

Typical properties of NSAIDs apparently not shared by Δ<sup>9</sup>-THC-11-oic acid are the so-called side effects; in particular, gastrointestinal and kidney toxicity. This statement is based partly on experimental findings and partly on anecdotal information. An example of the former is seen in Table 1, which shows the results from an acute ulcerogenicity test in rats (unpublished data). Δ<sup>8</sup>-THC-11-oic acid given at twice the maximal effective dose for the analgesia/anti-inflammatory assays produced zero lesions under conditions in which aspirin and indomethacin, two common NSAIDs, displayed significant toxicity. This finding is in agreement with the fact that chronic users of *Cannabis* who are exposed to high blood levels of this Δ<sup>9</sup>-THC metabolite appear to be free from NSAID-type toxicity. The precise basis for this difference is not well understood at this time; however, it may be partly due to a selective inhibition of COX-2 vs. COX-1 by Δ<sup>9</sup>-THC-11-oic acid similar to that shown by the synthetic CB acid 1',1'-dimethylheptyl-Δ<sup>9</sup>-THC-11-oic acid (DMH-THC-11-oic acid), which shows potent analgesic (Burstein *et al.*, 1998) and anti-inflammatory (Zurier *et al.*, 1998) actions (see Section 3). Selective inhibition of COX-2 has been suggested as a basis for NSAID activity free of gastrointestinal toxicity (Jouzeau *et al.*, 1997).

The findings described above strongly suggest that contrary to a long-held belief, the acid metabolites of Δ<sup>9</sup>-THC are not "inactive" and exhibit a pharmacological profile quite different from Δ<sup>9</sup>-THC. As a consequence of the former point of view, very little research has been done on a

<sup>1</sup>This refers to material that binds to anti-PGE in a radioimmunoassay procedure.

FIGURE 2. The major pathway of metabolism for  $\Delta^9$ -THC. Analogous pathways exist for  $\Delta^8$ -THC, cannabinol, and cannabidiol.



possible role for these metabolites in the overall effects of *Cannabis* use in humans. However, the acids may not only contribute to its analgesic properties, but could also attenuate the duration and intensity of the psychotropic effects. For example, in the mouse, it has been reported that the prior administration of  $\Delta^8$ -THC-11-oic acid limits the cataleptic response to  $\Delta^9$ -THC (Burstein *et al.*, 1987). This may be related to the observation that indomethacin, a potent NSAID, antagonizes specific responses to smoked marijuana in humans (Perez-Reyes *et al.*, 1991). If this “metabolite hypothesis” is valid, it could help explain the relatively low toxicity associated with THC, since the agonist would generate its own antagonist during the course of metabolism.

Time-course comparisons of  $\Delta^9$ -THC vs.  $\Delta^9$ -THC-11-oic acid have been carried out for both the hot plate (Burstein *et al.*, 1988) and PAF-induced edema models in the mouse (Burstein *et al.*, 1989). The findings in both cases showed an earlier and greater inhibition of response to the stimulus for the acid. These data are entirely consistent with the “metabolite hypothesis,” although other explanations such as different degrees of bioavailability for  $\Delta^9$ -THC compared with the acid are a possible cause for the more rapid and more robust action of  $\Delta^9$ -THC-11-oic acid. It is interesting to note that in the hot-plate study, THC actually caused hyperalgesia at early time points in contrast to the acid, which was antinociceptive at all time points.

### 3. SYNTHETIC ANALOGS

#### 3.1. General Considerations

More recently, studies on the CB acids have taken a somewhat different approach; namely, the use of more potent

synthetic analogs of  $\Delta^9$ -THC-11-oic acid as agonists. These analogs undoubtedly will provide better tools in the search for the sites of action of the acids in much the same way that CP55940 (the DMH analog of THC) has proven useful in characterizing the CB1 receptor. Moreover, it is expected that such acid analogs will be candidate molecules for testing as clinically useful agents free of the psychotropic effects of THC. An example of such an analog is DMH-THC-11-oic acid shown in Fig. 1. It has long been known that modification of the pentyl side chain in THC can produce molecules with altered potencies (Loev *et al.*, 1973). In particular, it has been found that increasing the chain length to 7 carbons and introducing branching close to the ring can lead to compounds with potencies that are 50–100 times that of THC. This strategy was employed in designing the structure of DMH-THC-11-oic acid, and the initial study supported the expectation for increased potency in several models of NSAID-type activity (Burstein *et al.*, 1992).

#### 3.2. Analgesic Effects

Further studies aimed at characterizing the possible analgesic potential of DMH-THC-11-oic acid have been carried out (Burstein *et al.*, 1998). Several tests in particular gave positive results in which the analog potently reduced behavioral responses to painful stimuli. In the phenylquinone writhing assay in the mouse, over the dose range of 0.2–10 mg/kg i.v., a complete reduction of the writhing response was obtained with an ID<sub>50</sub> of 1.24 mg/kg. In the mouse formalin antinociception test, a 64% reduction in the early-phase and a 48% reduction in the late-phase responses were observed at a dose of 4.64 mg/kg i.v. using three behavioral parameters for activity. This differs from a typical NSAID response, where only a late-phase effect would be expected. It is interesting to note that under these conditions, motor function was not altered, as seen in the rota rod procedure.

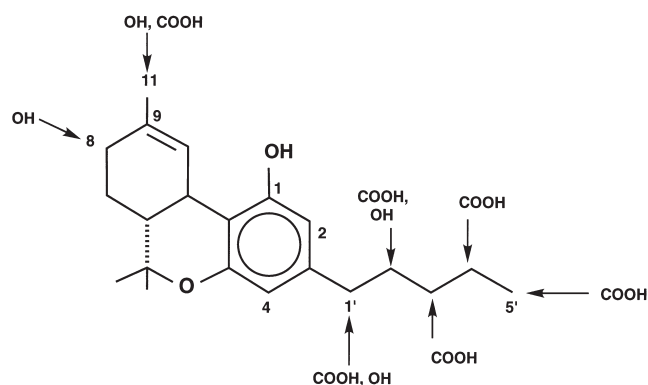


FIGURE 3. Carboxy metabolites of  $\Delta^9$ -THC. Arrows indicate positions of either hydroxylation or oxidation to a carboxy group.

TABLE 1. Acute Ulcerogenicity Test in Rats

Substance	Dose (mg/kg)	Incidence <sup>1</sup>	Mean score <sup>2</sup>
Aspirin	100	10/10	1.9
$\Delta^8$ -THC-11-oic acid	80	0/10	0.0
Indomethacin	20	6/11	0.77
Indomethacin	10	2/5	0.6

<sup>1</sup>Number of rats with lesions per total tested.

<sup>2</sup>Gastric mucosal lesions were scored on a scale of 0–3 according to size: 0, normal mucosa; 3, lesions greater than 5 mm in length.

In addition, hot-plate assays were done at two surface temperatures, 48°C and 58°C, to provide some insight into the mechanism of antinociceptive activity of the DMH-THC-11-oic acid. A significant decrease in potency was seen at the higher temperature, an effect that is considered characteristic of nonopioid receptor-mediated action.

Direct comparisons between DMH-THC-11-oic acid and morphine have yielded additional evidence that DMH-THC-11-oic acid is an effective analgesic in animal models (E. Dajani, personal communication). In two different tests, the mouse tail-clip and the mouse hot plate, at 55°C, DMH-THC-11-oic acid showed potencies comparable with morphine when measured at early time points. The long duration of action of DMH-THC-11-oic acid was revealed when the mice were tested in the tail-clip assay at later times, where it showed a considerably greater potency than morphine. This is in agreement with the duration of action seen in the adjuvant arthritis study, where it was found that administration of DMH-THC-11-oic acid was needed only on a thrice-weekly basis (Zurier *et al.*, 1998).

### 3.3. Inhibition of the Effects of Tetrahydrocannabinol

As was reported for  $\Delta^9$ -THC-11-oic acid (Burstein *et al.*, 1987), the DMH-THC-11-oic acid produced no cataleptic response in the mouse, as measured by the ring test (Fig. 4) (S. Burstein, W. Pearson and A. Zurier, unpublished data). Moreover, it was able to significantly reduce, in a dose-related manner, THC-induced catalepsy in the same model. The mechanism for this observed inhibition is a matter for speculation at this time; however, it seems improbable that the acids act directly on the ligand-binding site of the CB1 receptor. While little binding data is available for the analog, THC-11-oic acid does not appear to compete for sites with a known CB1 ligand (Compton *et al.*, 1993). Since  $\Delta^9$ -THC-induced catalepsy is thought to be mediated by the CB1 receptor, it seems likely that inhibition by the acids

may involve the attenuation of a downstream process following receptor-ligand interaction. There may even be some features in common with the analgesic/anti-inflammatory effects of the acids; for example, a reduction of eicosanoid synthesis since PGs can induce a cataleptic state when administered to mice (Burstein *et al.*, 1987). There is only weak binding of the acids to the CB2 receptor (Rhee *et al.*, 1997), which has been suggested to be involved in the mediation of anti-inflammatory effects (Facci *et al.*, 1995). Since most of the known CB1 ligands show significant affinity for CB2, the possibility is raised that the acids, which do not bind to CB1, may be ligands for a yet-to-be discovered CB3.

### 3.4. Anti-Inflammatory Effects

A long-standing experimental model for acute inflammation is the induction of leukocyte infiltration into a subcutaneous air pouch that approximates a synovial cavity. When DMH-THC-11-oic acid was tested in this model, a dramatic, dose-related decrease in pouch fluid cell count was observed (Zurier *et al.*, 1998). At a dose of 0.2 mg/kg/day *p.o.*, a 65% decrease in neutrophil infiltration occurred, demonstrating significant potency, as well as efficacy, of the analog in a clinically relevant model.

Further testing for anti-inflammatory action of DMH-THC-11-oic acid was done using the adjuvant-induced polyarthritis model in rats (Zurier *et al.*, 1998). This paradigm is considered to closely resemble rheumatoid arthritis in humans, and has been used widely in testing for anti-inflammatory activity in experiments with drug candidates. In this study, the analog was administered orally 3 times/week at a dose of 0.1 mg/kg, beginning 3 days after injection of Freund's complete adjuvant. The effects were evaluated both by clinical observations throughout the study and by histopathologic analysis of representative joint samples at the end of the study. The effects on one aspect of clinical symp-

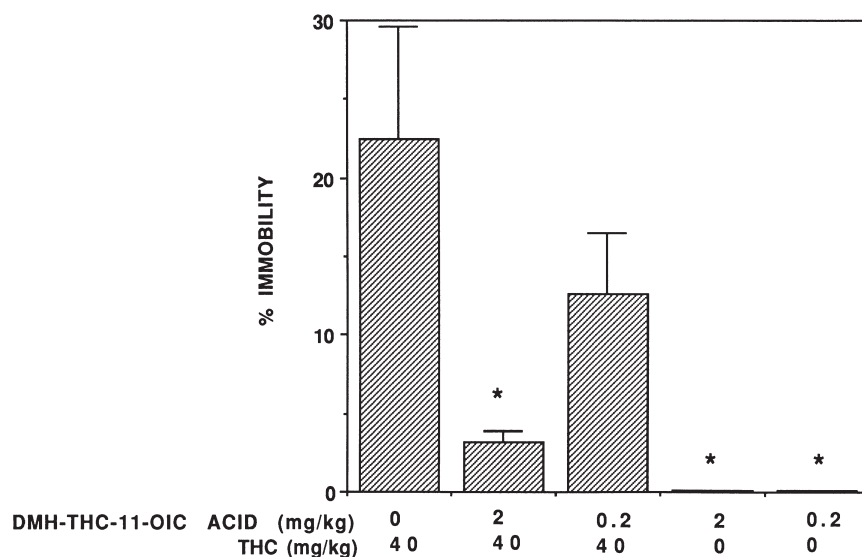


FIGURE 4. DMH-THC-11-oic acid inhibition of the cataleptic effect of THC in mice. DMH-THC-11-oic acid was given *p.o.* in 50 mL of peanut oil at doses of 0.2 and 2.0 mg/kg 120 min prior to testing.  $\Delta^9$ -THC (40 mg/kg) was given *p.o.* to all groups 60 min prior to testing. The ring test was performed as previously described (Burstein *et al.*, 1987). Values shown are the means from a group of 10 mice. \*P = 0.0001 by ANOVA (DMH-THC-11-oic acid vs. oil/ $\Delta^9$ -THC).

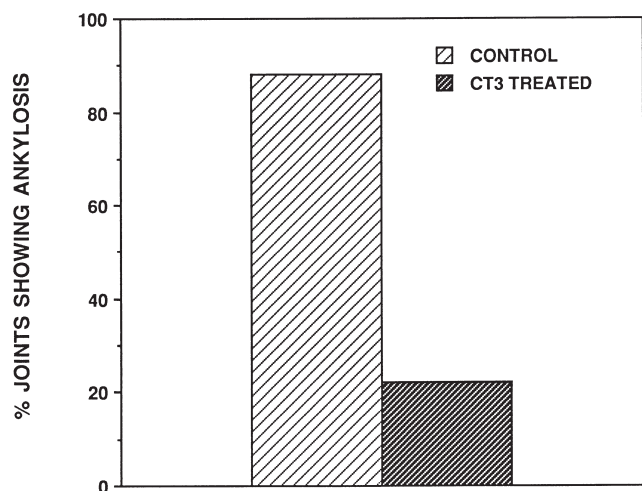


FIGURE 5. Reduction of permanent joint damage (ankylosis) by DMH-THC-11-oic acid. Lewis rats (120 g) obtained from Charles River Laboratories (Wilmington, MA) were treated with *Mycobacterium butyricum* in Freund's incomplete adjuvant. Each rat was given 2 mg *M. butyricum* in 0.2 mL by intradermal injection at the base of the tail. The data shown are the means from 9 rats, were obtained on day 35 of an adjuvant-induced arthritis study, and represent the number of joints with permanent damage. The rats had been treated with 0.1 mg/kg DMH-THC-11-oic acid each Monday, Wednesday, and Friday, beginning on day 3 after adjuvant injection. Adapted from Zurier *et al.* (1998).

tomology are shown in Fig. 5, which shows the effect of DMH-THC-11-oic acid on joint deformity (ankylosis) and demonstrates a significant improvement in drug-treated rats when compared with vehicle-treated controls. There is no evidence for the development of tolerance during the 30 days of drug administration; tolerance is a hallmark for the group of CBs such as THC with psychotropic activity. This latter point suggests a clear divergence in the mechanism of action between the CB acids and other CBs. The histopathologic findings were remarkable in that there appeared

to be a lack of permanent joint damage in the analog-treated animals (Zurier *et al.*, 1998). This was seen as minimal synovitis without pannus formation, in which cartilage, bone, and joint space appear intact. Further studies are needed to determine whether these effects are due solely to inhibition of eicosanoid synthesis or possibly involve the direct action of CT3 on other mediators of inflammation, such as metalloproteinases and cytokines.

#### 4. A POSSIBLE ANALOGY WITH ANANDAMIDE

A milestone in CB research occurred several years ago with the identification of a putative endogenous ligand for the CB1 receptor (Devane, 1994). The molecule called anandamide is the ethanolamide derivative of arachidonic acid (Fig. 6) and thus, may be considered a novel member of the eicosanoid family (Burstein *et al.*, 1995). Subsequent studies have led to the discovery of naturally occurring and synthetic analogs and metabolites of anandamide, suggesting that like THC, a family of biologically active substances may exist. Three examples of putative analogs/metabolites that have been synthesized are shown in Fig. 6. The structure labeled SB-1, which is the glycine derivative of arachidonic acid, is the anandamide counterpart of THC-11-oic acid. Computer-generated structural overlap studies of the corresponding hydroxy derivatives, i.e., anandamide and 11-hydroxy-DMH-THC, in general are in agreement with this concept (Burstein *et al.*, 1995). A preliminary report on some of the actions of these compounds was made recently (Burstein *et al.*, 1997) and the findings are shown in Fig. 7. Of interest is the observation that SB-1, like  $\Delta^9$ -THC-11-oic acid, shows relatively high activity in the hot-plate assay and low activity in the ring test. Structural specificity is demonstrated by the finding that SB-2, the bis homologue of SB-1, is inactive in both assays. Interestingly, SB-3 (*p*-arachidonylamido phenol) recently has been shown to be a selective inhibitor of anandamide transport in neuronal and glial cells (Beltramo *et al.*, 1997). This could possibly account for the modest effect it shows in the

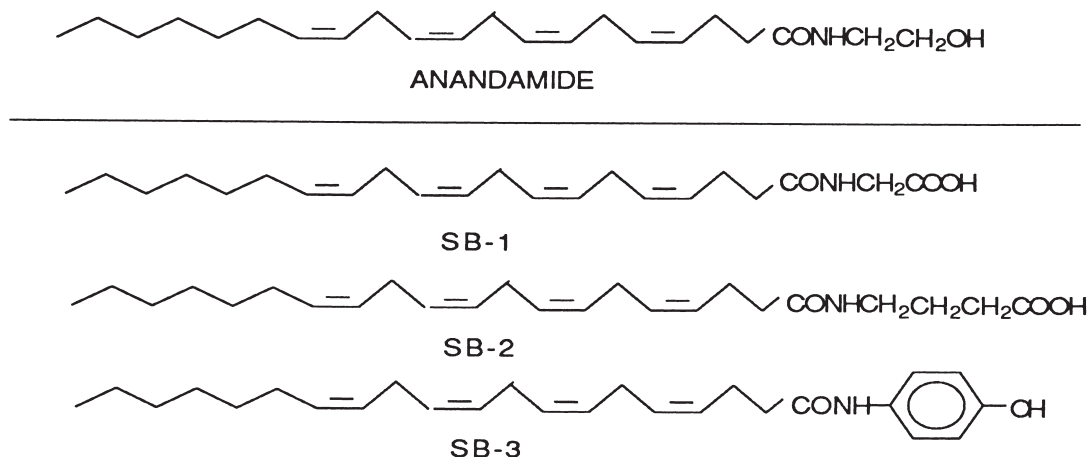


FIGURE 6. Structures of anandamide and several synthetic analogs.



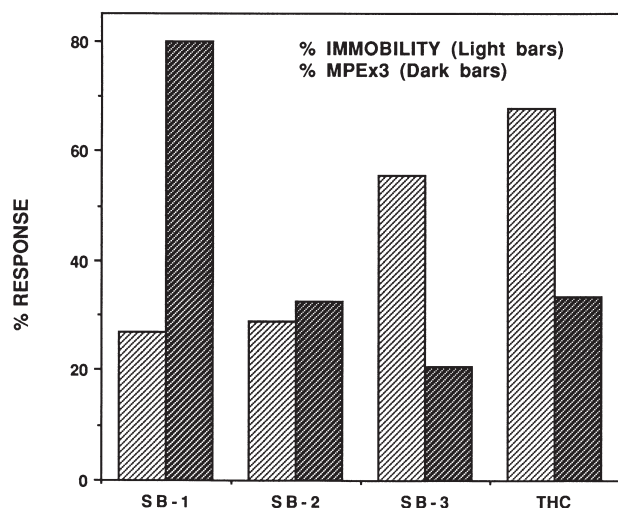


FIGURE 7. Effects of anandamide analogs in the hot-plate and ring tests compared with  $\Delta^9$ -THC. All substances were administered p.o. in peanut oil at a dose of 40 mg/kg 90 min prior to testing. The cutoff time for the hot plate was 30 sec and 300 sec for the ring test.  $N = 10$ . MPE, maximum possible effect.

ring test. Considering the potential importance of the anandamides, it would be of some interest to establish whether SB-1 is an endogenous member of this growing family of cell mediators.

## 5. THE QUESTION OF MECHANISM AND STRUCTURE-ACTIVITY CONSIDERATIONS

### 5.1. The Arachidonic Acid Cascade

Currently, a paucity of data is available describing the actions of the CB acids at the molecular level. What is known has been summarized in Fig. 8, which indicates specific points of inhibition located at COX-2 and 5-lipoxygenase (LOX) and has been reported previously (Burstein *et al.*, 1986a,b, 1989, 1992; Doyle *et al.*, 1990; Hunter *et al.*, 1984; Zurier *et al.*, 1998). No effects on phospholipases have been observed; however, this point has not been examined carefully. In contrast, the CBs with psychotropic activity, such as THC, appear to act as stimulators of arachidonic acid release by virtue of their actions on phospholipases (Audette *et al.*, 1991; Burstein and Hunter, 1978, 1981, 1995; Burstein *et al.*, 1982, 1983, 1984, 1985, 1994; Hunter *et al.*, 1986; Wartmann *et al.*, 1995). Moreover, THC shows only weak inhibitory action on COX-1 (Burstein and Raz, 1972), as do the other plant-derived CBs (Burstein *et al.*, 1973). The THC-induced release reaction has been shown to be mediated by either CB1 or CB2, depending on the cell type being studied (Hunter and Burstein, 1997), although this view has been questioned (Howlett, 1995a). This striking change in action on the arachidonic acid cascade in going from THC to its acid metabolite mirrors the changes in the *in vivo* responses described in Section 2.2, suggesting that the eicosanoid system plays a role in CB effects in general.

A possible explanation for CB-induced analgesia that involves effects on the arachidonic acid cascade can be de-

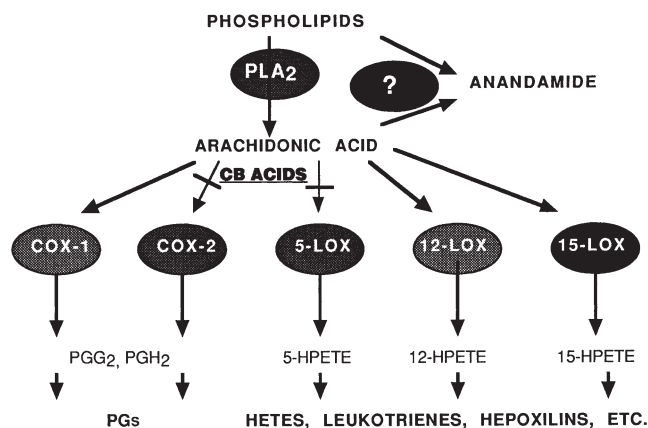


FIGURE 8. The arachidonic acid cascade—a possible site of action for the CB acids. The major pathways for the metabolism of arachidonic acid from phospholipid storage sites to the various eicosanoids. The inhibitory effects of the CB acids are indicated. PLA<sub>2</sub>, phospholipase A<sub>2</sub>; HETE, hydroxy-eicosatetraenoic acid.

rived from recent reports on the mechanism of opioid inhibition of  $\gamma$ -aminobutyric acid-mediated neurotransmission (Vaughan *et al.*, 1997; Williams, 1997). It was suggested that dual inhibition of COX and 5-LOX can greatly enhance the antinociceptive effects of opioids by shunting increased amounts of arachidonic acid through the 12-LOX pathway. In support of this hypothesis, it has been shown earlier that 12-hydroperoxy-eicosatetraenoic acid (HPETE), a product of this pathway, is a potent second messenger for presynaptic inhibition of *Aplysia* sensory cells (Piomelli *et al.*, 1987). The CB acids inhibit both COX and 5-LOX, an effect that might result in elevated levels of 12-LOX metabolites at presynaptic sites, thereby enhancing the effects of both endogenous and exogenous opioids. The CB acids, therefore, may act on the opioid pathway downstream of the receptor and thus, their actions would not be naloxone-sensitive. It is interesting to note that primary CBs such as THC can elevate free arachidonic acid levels in a wide range of experimental models (Burstein, 1992), and this effect appears to be either CB1 or CB2 receptor-mediated, depending on the cell type studied (Hunter and Burstein, 1997). This effect could also result in increased synthesis of 12-LOX metabolites, especially *in vivo*, where simultaneous metabolism to the CB acids occurs. This hypothesis would provide a possible explanation for the findings of Lichtman and Martin (1997) showing that SR141716A, a CB1 antagonist, reduces CB-induced antinociception in rats. Thus, the primary CBs and the acid metabolites exert their analgesic actions at different points on the same pathway, with both resulting in an elevation of 12-LOX products. These will be interesting points to confirm experimentally and may help resolve outstanding issues concerning the mechanism of CB-induced analgesia.

### 5.2. Other Systems

The most widely studied mechanism for receptor-mediated CB action has been the ability to inhibit hormone-stimu-

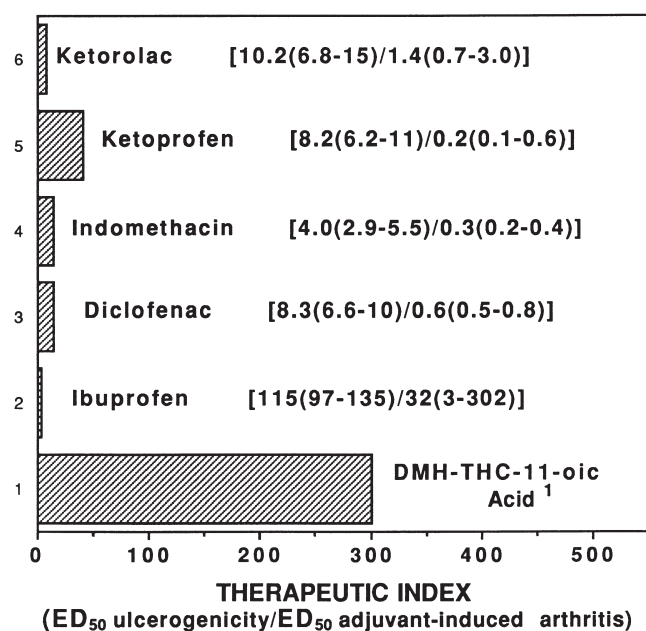


FIGURE 9. Therapeutic index of DMH-THC-11-oic acid compared with several NSAIDs. The therapeutic indices are adapted from Young and Yee (1994) and were calculated from the ratio of the  $ED_{50}$  ulcerogenicity/ $ED_{50}$  adjuvant-induced arthritis in the rat (95% confidence limits). <sup>1</sup>The  $ED_{50}$  values for DMH-THC-11-oic acid are estimates since a dose of up to 30 mg/kg/day did not produce detectable ulceration (E. Dajani, unpublished data). The  $ED_{50}$  in the rat adjuvant arthritis test for DMH-THC-11-oic acid is also estimated from the available data (Zurier *et al.*, 1998).

lated adenylate cyclase *in vitro* (for a recent review, see Pertwee, 1997). There is no evidence that the CB acids directly affect this system; however, there may be indirect effects due to their ability to inhibit PG synthesis. It has been suggested that CB modulation of adenylate cyclase may be a result of their effects on eicosanoid levels (Hillard and Bloom, 1983; Burstein *et al.*, 1995). Thus, it is possible that the acids may down-regulate this response by inhibiting the activity of COX.

Effects on anandamide levels by the acids represent another possible mechanism of action. The NSAID ibuprofen has been reported to inhibit the hydrolytic metabolism of anandamide (Fowler *et al.*, 1997), raising the possibility that the acids may also produce such an effect. In addition, it has been reported that anandamide is a substrate for COX-2 (Yu *et al.*, 1997), so that COX-2 inhibitors such as the acids may cause an elevation of cellular anandamide by shutting down this route of metabolism. A similar observation on the elevation of anandamide levels in calcium ionophore-challenged RAW264.7 cells by indomethacin supports this hypothesis (Pestonjasp and Burstein, 1998).

### 5.3. Structure-Activity Considerations

Most of the comprehensive reports and reviews on CB structure-activity relationships have not included data on the CB acids (Loev *et al.*, 1973; Wilson and May, 1975; Johnson *et al.*, 1982; Razdan, 1986; Rapaka and Makriyan-

nis, 1987; Howlett *et al.*, 1988; Little *et al.*, 1988; Reggio *et al.*, 1989; Makriyannis and Rapaka, 1990; Compton *et al.*, 1991, 1992a,b, 1993; Thomas *et al.*, 1991; Melvin *et al.*, 1993, 1995; Adams *et al.*, 1995; Martin *et al.*, 1995; Howlett, 1995b; Pertwee, 1997). The general picture that has emerged from all of these papers suggests that there are three important regions in the THC structure for so-called cannabimimetic activity. This term has come to mean substances with significant affinity for CB1. The three regions are the side chain (R1, Fig. 1), the phenolic hydroxyl, and the substituent at position 9 (R2, Fig. 1). The position of the double bond (8,9 or 9,10) has only a minor effect on CB1 binding; however, the stereochemistry of the ring juncture is critical. Any departure from the R,R absolute configuration shown in Fig. 1 leads to a decrease in cannabimimetic activity. Modification of the side chain from the *n*-pentyl can result in a change in potency that in some cases, leads to dramatic increases in activity. This seems to apply not only to psychotropic activity, but also to analgesic and anti-inflammatory actions as well. This side-chain effect appears to be quite general and seems to apply to CB interactions with other sites, in addition to CB1.

Changes in activity involving the nature of the substituent at position 9 were originally observed in studies on the metabolites of THC (Section 2). Conversion to a hydroxymethyl generally results in derivatives with potencies equal to or greater than the corresponding methyl derivative. However, further conversion to a carboxylated analog causes a profound alteration in the pharmacological profile in which there is a dramatic decrease in psychotropic activity. These carboxy derivatives, on the other hand, retain the full analgesic and anti-inflammatory activities of the parent molecules (Burstein *et al.*, 1986a, 1987, 1988, 1989; Watanabe *et al.*, 1980). Martin *et al.* (1991) have examined this phenomenon as part of an extensive structure-activity relationship study using a battery of behavioral assays in mice to quantitate activity. They reported their findings as an average potency over 4 assays relative to THC for changes in spontaneous activity, hypothermia, antinociception, and catalepsy. Working in the  $\Delta^9$  DMH series, they found a 70-fold decrease in average relative potency in going from the 11-hydroxy to the 11-carboxy derivative; however, the acid still was 2.6 times more "active" than THC. Unfortunately, they did not report data for the individual assays, so it is possible that the acid was totally inactive in the catalepsy test, but still very active in the assay for antinociception, which, if this were the case, would compare well with the findings from other laboratories (Burstein *et al.*, 1988; Watanabe *et al.*, 1980). It should be noted that the potency value reported by Martin *et al.* (1991) is approximately 100-fold less than that reported by Zurier *et al.* (1998) in their rat adjuvant arthritis study and about 10-fold lower than that reported by Burstein *et al.* (1998) for inhibition in a mouse writhing assay.

The underlying reasons for the low psychotropic potencies, i.e., lack of binding to CB1, of the CB acids are a matter of speculation, given the limited data that are available

(Rhee *et al.*, 1997). One possibility could be differences in steric volume of the methyl group compared with the carboxy group. Calculations such as those done by Reggio *et al.* (1993) for a series of CBs that would include examples of the acids might support such a contention. A second possibility may have something to do with electronic properties, as has been suggested by Da Silva and Trsic (1995). They have reported a correlation between LUMO energies and psychotropic activity for a series of 7 CBs that includes THC-11-oic acid. Of course, the most interesting question remains; namely, if the acids do not bind to CB1, with which sites do they interact?

## 6. SUMMARY

The natural CB acids are a large and structurally diverse group of substances that are produced *in vivo* following the ingestion of the primary CBs. Tissue levels of these metabolites persist long after the decline in the psychotropic effects, suggesting that they do not share these properties with  $\Delta^9$ -THC. This is supported by observations in both experimental animal models (Burstein *et al.*, 1987; Watanabe *et al.*, 1980) and in humans (Perez-Reyes, 1985), where cannabimimetic activity was found to be absent. Moreover, they very likely antagonize some of the effects of  $\Delta^9$ -THC, as evidenced by their ability to reduce its cataleptic action in mice (Burstein *et al.*, 1987) (Fig. 4). This antagonism probably is not due to competition for binding to the CB1 receptor, but may be related to their ability to inhibit eicosanoid synthesis; however, the precise mechanism could include effects on other systems as well. The attenuating effects of the acids present an interesting situation where the agonist  $\Delta^9$ -THC produces increasing levels of inhibitors, the CB acids, with time. Thus, the use of *Cannabis* would seem to have a built-in safety mechanism, provided the subject has an adequate rate of metabolism.

Synthetically derived CB acids can provide potentially useful candidate molecules for drug development. This strategy is based on reports demonstrating that the natural acids exhibit NSAID-like activity in mice at doses comparable with those at which  $\Delta^9$ -THC is active (Burstein *et al.*, 1988, 1989; Doyle *et al.*, 1990). An example of a synthetic acid, DMH-THC-11-oic acid, that shows potent analgesic (Burstein *et al.*, 1998) and anti-inflammatory (Burstein *et al.*, 1992; Zurier *et al.*, 1998) activity in animal models has been described in this review. In addition to showing analgesic potencies comparable with or greater than morphine in animals (see Section 3.2), it was highly effective in reducing leukocyte migration and in preventing permanent joint damage in the rat adjuvant-induced arthritis model (Zurier *et al.*, 1998). Of particular interest are the observations that both the natural (Table 1) and the synthetic acids (E. Dajani, personal communication) appear to lack the ulcerogenic actions that are often associated with many of the NSAIDs currently in clinical use. A comparison of DMH-THC-11-oic acid with several well-known NSAIDs is shown in Fig. 9 (E. Dajani, personal communication).

The therapeutic indices for the drugs shown were based on the ED<sub>50</sub> values for their effects in the rat adjuvant arthritis and acute ulcerogenicity models. There is a greater than 7-fold more favorable index with DMH-THC-11-oic acid when compared with ketoprofen, the best example of the NSAIDs; ibuprofen, the worst example, was more than 80-fold less favorable than DMH-THC-11-oic acid. If these findings hold up in the clinic, the CB acids may provide a breakthrough in the long-sought goal of finding a medicinal CB free of psychotropic activity.

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