

Antagonism to the Actions of Platelet Activating Factor by a Nonpsychoactive Cannabinoid¹

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ABSTRACT

A recent report from our laboratory gave evidence showing that tetrahydrocannabinol (THC)-7-oic acid has antinociceptive activity in the mouse. We also pointed out that this substance, which is a major metabolite of THC in most species including humans, is probably responsible for the well known analgesic properties of the parent drug. The present report contains findings that suggest THC-7-oic acid may have considerable activity as an antagonist to platelet activating factor, which may also explain the known properties of THC as a bronchodilator, antipyretic and antirheumatic agent. In the mouse ear edema test, THC-7-

oic acid appeared to be about as efficacious as phenidone; however, its potency was less than either phenidone or indomethacin. When compared with the parent drug, THC, in the platelet activating factor-induced paw edema assay, it acted more quickly and produced a greater reduction of edema. This is consistent with the possibility that THC must be metabolized to the 7-oic acid for activity to be seen. Its activity in preventing platelet activating factor-induced mortality was comparable to naproxen. *In vitro* studies suggest that THC-7-oic acid can inhibit both cyclooxygenase and 5-lipoxygenase activities in intact cells.

Cannabinoids are most widely known for their psychotropic effects; however, they also display a variety of other activities, some of which have potential therapeutic application. For example, we have recently reported that the major terminal metabolite of Δ^1 -THC, THC-7-oic acid (fig 1), has antinociceptive properties (Burstein *et al.*, 1988). We gave evidence that this metabolite seems to be the agent responsible for the numerous reports concerning the analgesic effects of THC and that it probably acts by suppressing eicosanoid production in a manner analogous to the nonsteroidal anti-inflammatory agents.

The ability of THC-7-oic acid to reduce eicosanoid synthesis can be directly observed in other experimental models. Some time ago, we showed that THC stimulates the cyclooxygenase pathway in WI-38 fibroblasts (Burstein *et al.*, 1983) and the lipoxygenase pathway (Burstein *et al.*, 1984) in mouse peritoneal cells in culture. When the WI-38 cells were pretreated with THC-7-oic acid, the synthesis of PGs was inhibited in a noncompetitive manner (Burstein *et al.*, 1986). This antagonism of THC, which seems to be functional in nature, could be demonstrated *in vivo* using the model for catalepsy called the

"ring test" (Burstein *et al.*, 1987). Prior administration of THC-7-oic acid to mice, in doses equivalent to the THC, reduced the cataleptic response of the latter by one-half. This finding was consistent with the report by Fairbairn and Pickens (1979), where it was shown that other inhibitors of eicosanoid synthesis could likewise reduce the cataleptic effect of THC.

Certain activities of THC, such as its bronchodilatory properties (Tashkin *et al.*, 1973; Williams *et al.*, 1976), suggest that THC-7-oic acid may be responsible for this effect as well. PAF is believed to be an important mediator of bronchoconstriction such as that produced during an asthmatic crisis (Barnes *et al.*, 1988). Thus, antagonism to the effects of PAF has been thought to be a possible mechanism of action for certain antiasthmatic agents. This has prompted us to investigate the possibility that THC-7-oic acid may be an effective antagonist to the actions of PAF and that this may be the basis for its bronchodilatory action.

Methods

Chemicals. The cannabinoids were obtained from the National Institute on Drug Abuse (Rockville, MD) and their purity was monitored by reversed phase high pressure liquid chromatography. Calcium ionophore, indomethacin, phenidone, arachidonic acid, naproxen and PAF were purchased from Sigma Chemical Co. (St. Louis, MO). [1-¹⁴C]Arachidonic acid (specific activity, 52.7 Ci/mmol) was purchased from New England Nuclear Corp. (Boston, MA). ($\pm$)-5-HETE was

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ABBREVIATIONS: THC, tetrahydrocannabinol; PG, prostaglandin; PAF, platelet activating factor; HETE, hydroxyeicosatetraenoic acid; MEM, minimal essential medium; NDGA, nordihydroguaiaretic acid.

purchased from Biomol Research, Inc. (Plymouth Meeting, PA). The vehicle was "Planters" pure peanut oil.

PAF-induced paw edema. Our conditions were based on the method published by Calhoun *et al.* (1987), except that we used the technique of Hall and Hallett (1975) for measuring paw volume. Water was substituted for mercury as the displacement medium and was found to give satisfactory results. PAF (1.0 μ g) dissolved in 50 μ l of 5% ethanol in saline was injected s.c. into the plantar surface of the right hind paw of CD-1 female mice (20–25 g) obtained from Charles River Labs. The mice were under ether anesthesia during this procedure. The volume of the right foot was measured to the level of the lateral malleolus by water displacement before treatment and 15 min after PAF injection. The change in paw volume was calculated for each mouse and the significance for each group was determined by a paired *t* test analysis.

Ear edema test. Female CD-1 mice (20–25 g) were treated with topically applied arachidonic acid (0.5 mg in 10 μ l ethanol) on the inner surface of the ear. One hour later the animals were sacrificed by cervical dislocation and the ears removed, trimmed to give uniform areas and the wet weights obtained. In a control study, mice received ethanol only on the right ear and arachidonic acid in ethanol on the left ear. An increase in mean ear weight from 25.2 ± 3.3 mg (right ear) to 52.2 ± 7.8 mg (left ear) or 107% was observed; its significance ($P = .011$) was calculated using a paired *t* test analysis. Drug effects were measured by applying the compound in ethanol (10 μ l) to the left ear; after 1 hr, arachidonic acid in ethanol was applied and the test completed as above.

PAF-induced mortality. The drugs dissolved in peanut oil (50 μ l) or the vehicle were administered orally to female CD-1 mice (20–25 g). One hour later PAF (10 μ g) dissolved in 5% ethanol-saline (0.1 ml) was injected i.p. and the mortality in each group noted at 1, 2 and 24 hr. The significance of each observation was determined by comparison to the vehicle-treated group using the χ^2 test.

Inhibition of PG and 5-HETE synthesis. Peritoneal cells were collected from female CD-1 mice (20–25 g) after sacrifice by cervical dislocation. The cells from each group of mice were pooled, centrifuged and suspended in MEM. Aliquots containing 4.5×10^6 cells in 3.0 ml of MEM were placed in culture dishes and incubated for 18 hr at 37°C. By this procedure, a relatively homogenous monolayer of macrophages was obtained. Eicosanoid synthesis was brought about by the addition of [14 C]arachidonic acid (2×10^5 dpm; 0.22 μ M) followed by a calcium ionophore, A23187 (0.03 μ M). After 15 min, the reaction was stopped by the addition of citric acid to pH 4 and the products were extracted with ethyl acetate. Silica gel thin layer chromatography (hexane:ether:acetic acid; 50:50:1) was used to separate the PGE₂, PGF_{2 α} and 5-HETE, which were identified by comparison with authentic materials (Salmon and Flower, 1982). Carbon-14 content was measured by liquid scintillation counting of the eicosanoid-containing zones. The significance of drug-induced changes was calculated using the Student's *t* test when compared with vehicle-treated (ethanol, 30 μ l) cells.

Results

The s.c. injection of PAF results in the development of measurable edema at the site of injection. This effect has been

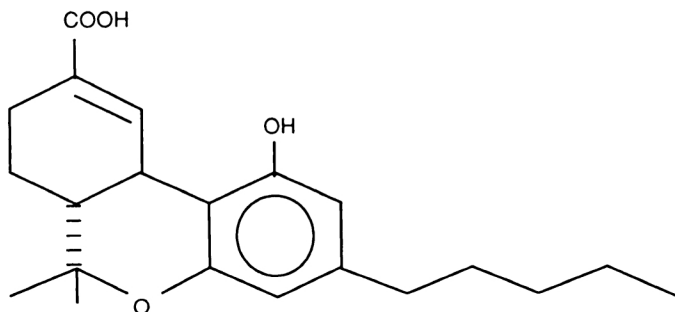


Fig. 1. THC-7-oic acid.

utilized in an assay procedure for the evaluation of potential anti-inflammatory agents (Calhoun *et al.*, 1987). We have applied this method to our study of the therapeutic activity of THC-7-oic and the results are shown in figures 2 and 3. Oral administration in peanut oil of THC-7-oic (20 mg/kg) gave a peak response at 1 hr; the parent drug, THC, gave a weaker response and required 3 hr to reach its maximum effect when given under the same conditions. A comparison of the two cannabinoids at several doses again showed the metabolite to be slightly more active than THC when each was studied at its optimal time interval. At a dose of 20 mg/kg, THC-7-oic acid

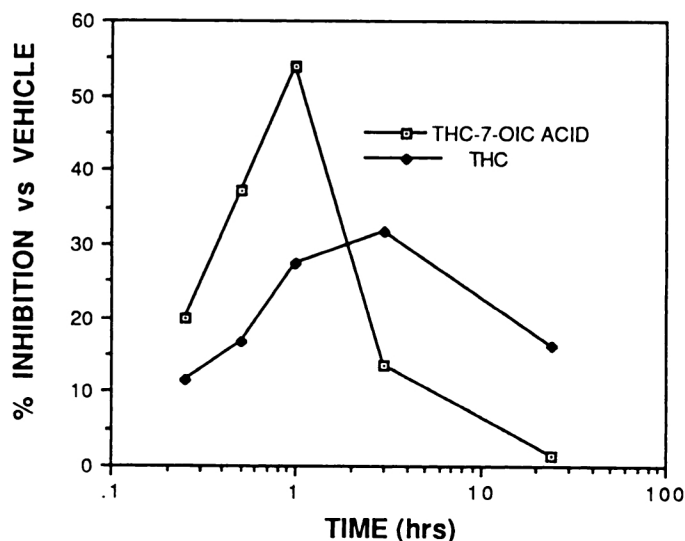


Fig. 2. PAF-induced edema-time course. The drugs were given p.o. to CD-1 female mice (20–25 g) in peanut oil (50 μ l) at a dose of 20 mg/kg. At the indicated time, 1.0 μ g of PAF (Sigma) in 50 μ l of 5% ethanol in saline was injected s.c. into the plantar surface of the right hind paw under ether anesthesia. The volume of the injected foot to the level of the lateral malleolus was measured by water displacement just before drug administration and again 15 min after PAF injection. Using groups of 10 mice, a significant change in paw volume ($P < .001$) was obtained in each case. Vehicle controls gave volume increases of 31 to 36%.

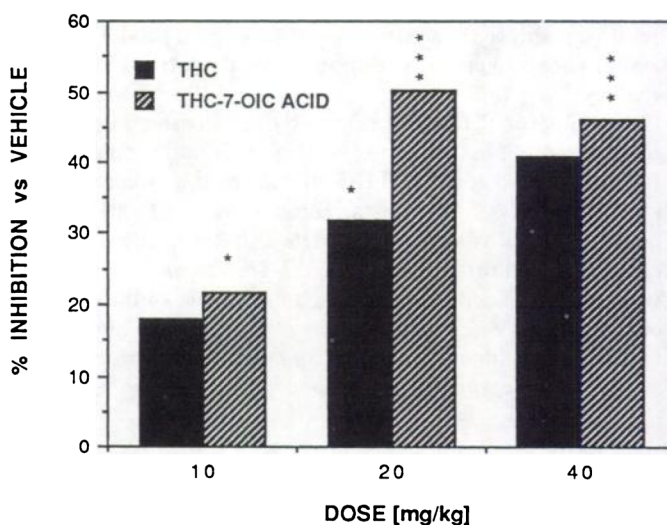


Fig. 3. PAF edema dose-response. The experiments were carried out as described under "Methods." THC was given 3 hr before PAF, whereas THC-7-oic acid was given 1 hr before the PAF injection. The drug doses were as indicated. * $P < .05$; *** $P < .001$ for drug- vs. vehicle-treated mice ($N = 10$).

was significantly more active than THC at the same dose when compared by the Student's *t* test ($P < .05$).

PAF-induced mortality in the mouse has been proposed as a model for allergy and anaphylactic shock because the effects of PAF in this species do not appear to be due to platelet aggregation (Meyers *et al.*, 1983; Young *et al.*, 1985). Figure 4 shows a PAF mortality dose-response relationship obtained under our experimental conditions. Based on these results, we chose a dose of 10 μ g of PAF per mouse for our subsequent studies inasmuch as this produced a survival rate of around 32%. The results shown in table 1 demonstrate that at 40 mg/kg THC-7-oic acid is an effective agent in preventing PAF-induced mortality. Naproxen at the same dose provided less protection, whereas phenidone had no beneficial effect.

Earlier studies demonstrated that THC-7-oic acid could inhibit eicosanoid synthesis by the cyclooxygenase pathway (Burststein *et al.*, 1986). In figure 5 we now report evidence that this cannabinoid is able to reduce product formation by 5-lipoxygenase. Over a dose range of 3.2 to 8 μ M, 5-HETE synthesis by mouse peritoneal cells was inhibited up to 50%. In the same system, PGE₂ synthesis was inhibited by 35%, whereas the production of PGF_{2 α} was reduced by 43% (fig. 6A). NDGA at a dose of 32 μ M lowered 5-HETE synthesis by about 39% under the conditions of our experiment (fig. 6B).

Topical application of arachidonic acid to the mouse ear produces a transient edema in which the synthesis of cyclooxygenase and lipoxygenase products plays a major role (Opas *et al.*, 1985). We compared THC-7-oic acid to naproxen, indomethacin and phenidone in this type of test and the results are contained in figure 7. Naproxen and indomethacin are considered to be cyclooxygenase inhibitors, whereas phenidone is believed to be a dual inhibitor in this system (Young *et al.*, 1984). Our results were similar to those published by Young *et al.* (1984), suggesting that our model was operating in a comparable manner to theirs. Indomethacin and phenidone seem to be more effective than THC-7-oic acid; however, it appears to have greater antiedema activity than naproxen.

Discussion

A previous report by Sophia *et al.* (1973) described the antiedema activity of THC. On the other hand, data from our

laboratory showing that THC caused the release of arachidonic acid followed by increased eicosanoid production (Burststein *et al.*, 1983) seem to be contrary to the above report on its effects on experimental edema. Our recent discovery that THC-7-oic acid, the principle metabolite of THC, has the opposite effect, namely inhibition of eicosanoid synthesis (Burststein *et al.*, 1986), provides an explanation for this paradox. A prediction of this hypothesis is that the metabolite should have a more rapid antiedema time course and possibly a more potent effect than the parent drug. These were, in fact, our observations which are shown in figures 2 and 3. In addition to its action against PAF-induced edema, the metabolite was highly effective in reducing carrageenan-induced edema (Burststein, 1988).

The above findings agree with those which we recently reported on the antinociceptive effects of THC (Burststein *et al.*, 1988). In that study, we compared THC-7-oic acid with THC in the mouse hot-plate assay. At early time points, THC actually shortened the thermal response time and only at 30 min did the characteristic increase in latency begin to appear. By contrast, THC-7-oic acid was antinociceptive at all times and was generally more potent than the parent drug. Thus a pattern seems to be emerging in which some of the actions produced by exposure to THC are probably due to its transformation to THC-7-oic acid.

The reduction of PAF-induced edema is thought to be indicative of "dual inhibition," namely, a decrease in lipoxygenase as well as cyclooxygenase activity (Calhoun *et al.*, 1987). It has been suggested that the mouse ear edema assay is also a test for dual inhibition (Opas *et al.*, 1985). This is based on observations that the products of both pathways can be detected under the conditions of this assay. As shown by the data in figure 7, the acid metabolite is able to reduce the edematous response to arachidonic acid in the mouse ear.

Another effect of THC for which no convincing explanation has been offered is its bronchodilatory action. Again, it is difficult to reconcile this type of activity with our earlier observations that THC increases the activities of phospholipases. PAF-induced mortality in the mouse has been suggested as a model for systemic anaphylaxis and the potent bronchoconstrictor action of PAF is well known. In table 1, data are shown which demonstrate that THC-7-oic acid effectively an-

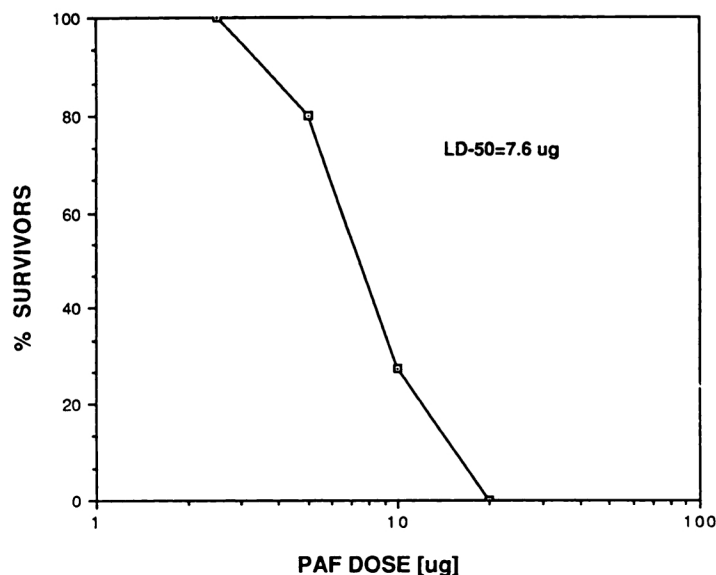


Fig. 4. PAF mortality. Female CD-1 mice (20–25 g) were injected i.p. with PAF (Sigma) dissolved in 5% ethanol-saline at the indicated doses. Groups of five mice were used and the data shown indicate survival at 1 hr after PAF injection. Significance by the χ^2 test; $P < .0005$.

TABLE 1
PAF-induced mortality^a

Treatment ^a	Dose mg/kg	Survivors/N ^c		
		1 hr	2 hr	24 hr
Vehicle		8/25 (32)	8/25 (32)	8/25 (32)
THC-7-oic acid	80	7/10 (70)*	7/10 (70)*	7/10 (70)*
THC-7-oic acid	40	12/15 (80)*	12/15 (80)*	12/15 (80)*
THC-7-oic acid	20	5/20 (25)	5/20 (25)	5/20 (25)
Phenidone	40	1/5 (20)	1/5 (20)	1/5 (20)
Naproxen	40	5/5 (100)*	3/5 (60)	3/5 (60)
Naproxen	20	4/10 (40)	4/10 (40)	2/10 (20)

^a Female CD-1 mice (20–25 g) were injected i.p. with PAF (Sigma; 10 µg) dissolved in 5% ethanol-saline.

^b All drugs were administered p.o. in peanut oil (50 µl) 1 hr before PAF injection.

^c N = total number of mice treated; values are percent survivors.

* Significant at greater than 95% level using the χ^2 test when compared to vehicle.

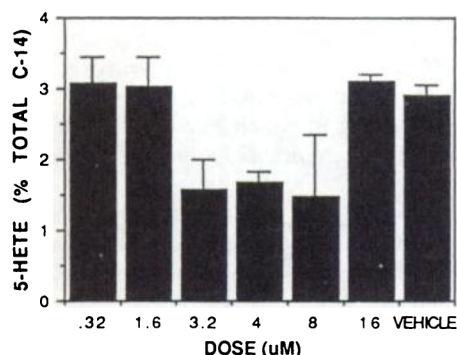


Fig. 5. Inhibition of 5-HETE synthesis. Unstimulated peritoneal cells from female CD-1 mice were allowed to adhere overnight to plastic culture dishes. The adhering cells (4.5×10^6) were then challenged with A23187 (0.03 µM) in the presence of [14 C]arachidonate (0.22 µM) for 15 min. The THC-7-oic acid was added 15 min before the A23187. The 5-HETE was analyzed by thin layer chromatography and is reported as percent of the total recovered carbon-14 $\pm$ S.D. Concentrations between 3.2 µM and 8 µM gave significant changes when compared to the vehicle ($P < .03$).

tagonizes PAF-induced mortality. We do not believe that THC-7-oic acid is a PAF receptor antagonist; however, there is no direct evidence available on this point.

Prior reports suggest that the acid is an inhibitor of the cyclooxygenase pathway for arachidonic acid metabolism. Evidence that it is also a 5-lipoxygenase inhibitor is shown in figure 5. A significant reduction in 5-HETE synthesis by calcium ionophore-stimulated mouse peritoneal cells was observed at doses from 3.2 to 8 µM. At a 16 µM dose, the level of 5-HETE returned to the vehicle control value. There is a suggestion of a similar type of dose-response relationship in the PAF mortality test (table 1) and also in the results we obtained in the mouse ear edema test (fig. 7). Such biphasic or bell-shaped dose-response relationships for cannabinoid effects have been reported in a variety of systems (Hershtkowitz *et al.*, 1977; Hillard and Bloom, 1982, 1983; Burstein *et al.*, 1988; Howlett and Fleming, 1984; Holmes *et al.*, 1986). As yet, there has been no clear explanation for what seems to be a frequently observed feature of cannabinoid-induced responses.

Despite its long history as a therapeutic drug, cannabis and its constituents have had limited use in modern medicine because of the psychoactivity associated with cannabinoids (Mechoulam, 1986). Thus, an important goal for medicinal chemists in this area has been the search for cannabinoids

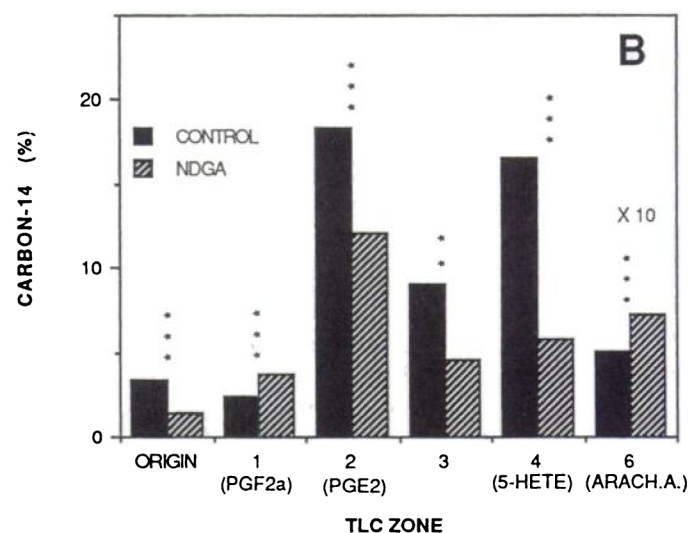
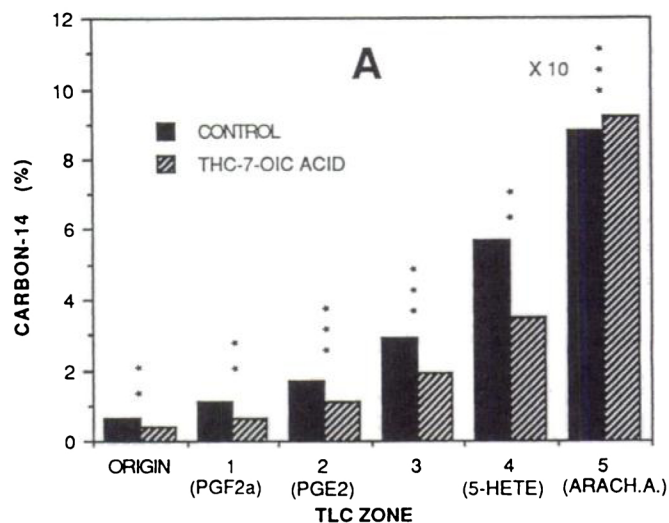


Fig. 6. Inhibition of eicosanoid synthesis. The conditions were as in figure 5. A: THC-7-oic acid concentration, 3.2 µM. B: NDGA concentration, 32 µM. Controls were vehicle-treated cells (30 µl of ethanol). ** $P < .01$; *** $P < .0001$ for drug- vs. vehicle-treated cells ($N = 3$).

which retain activities such as analgesia, bronchodilation, reduction of edema, etc., but have minimal effects on mood or behavior. The findings discussed in this report suggest that THC-7-oic acid may be a good candidate for such an agent because earlier publications demonstrated that it appears to be totally devoid of psychoactivity (Mechoulam *et al.*, 1973; Watanabe *et al.*, 1980; Perez-Reyes, 1985; Burstein *et al.*, 1988). Moreover, by virtue of the fact that THC-7-oic acid is the major metabolite of THC, a sizeable human population has received chronic exposure to this cannabinoid. The absence of reports in the literature on toxic effects such as those related to the widely used antiinflammatory agents leads us to predict that THC-7-oic acid is a relatively safe drug. Direct testing would, of course, be needed to establish this point.

Finally, it is interesting to note how the relatively small change in structure in going from THC to THC-7-oic acid produces such a profound change in pharmacological activity. This is more remarkable when one considers that the intermediate metabolites, 7-hydroxy and 7-oxo-THC, are pharmacologically similar to THC, suggesting that this change in properties occurs between the aldehyde and acid. The carbox-

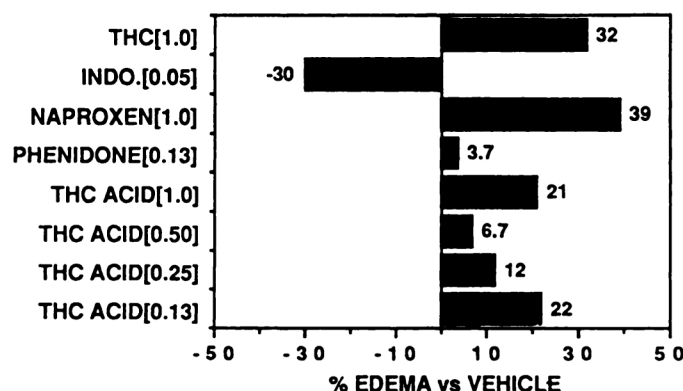


Fig. 7. Arachidonate-induced ear edema. The drugs were dissolved in ethanol (10 μ l) and applied to the inner surface of the left ear of female CD-1 mice (20–25 g); group sizes were from 5 to 10 mice. After 1 hr arachidonic acid (0.5 mg in 10 μ l of ethanol) was applied to the same area for an additional period of 1 hr. The mice were then sacrificed and the ear weights obtained. Controls of arachidonate vs. vehicle showed an increase in weight from a mean of 25.2 ± 3.3 to 52.2 ± 7.8 mg. All of the above drug treatment values were significant at greater than 95% by analysis of variance when compared to the arachidonate control ($P = .0001$). The doses are shown in brackets (milligrams/ear).

ylic acid group seems to be required for the activities reported here and leads us to suggest that other cannabinoid derivatives containing this structural feature would also produce effects of potential therapeutic value.

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