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ANALGESIC AND ANTIINFLAMMATORY ACTIVITY OF CONSTITUENTS OF *Cannabis sativa* L.

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Abstract—Two extacts of *Cannabis sativa* herb, one being cannabinoid-free (ethanol) and the other containing the cannabinoids (petroleum), were shown to inhibit PBQ-induced writhing in mouse when given orally and also to antagonize tetradecanoylphorbol acetate (TPA) -induced erythema of mouse skin when applied topically. With the exception of cannabinol (CBN) and Δ^1-tetrahydrocannabinol (Δ^1-THC), the cannabinoids and olivetol (their biosynthetic precursor) demonstrated activity in the PBQ test exhibiting their maximal effect at doses of about 100 μg/kg. Δ^1-THC only became maximally effective in doses of 10 mg/kg. This higher dose corresponded to that which induced catalepsy and is indicative of a central action. CBN demonstrated little activity and even at doses in excess of 10 mg/kg could only produce a 40% inhibition of PBQ-induced writhing. Cannabidiol (CBD) was the most effective of the cannabinoids at doses of 100 μg/kg. Doses of cannabinoids that were effective in the analgesic test orally were used topically to antagonize TPA-induced erythema of skin. The fact that Δ^1-THC and CBN were the least effective in this test suggests a structural relationship between analgesic activity and antiinflammatory activity among the cannabinoids related to their peripheral actions and separate from the central effects of Δ^1-THC.

INTRODUCTION

Various preparations of *Cannabis sativa* have been employed for their medicinal effects, including antipyretic, antirheumatic, antiallergic, and analgesic purposes (1). Extracts of *Cannabis* have been shown to possess analgesic activity (2, 3), and Δ^1-tetrahydrocannabinol (Δ^1-THC), the psychoactive component of *Cannabis* has also been shown to possess this activity in various models (4–6). In addition, cannabinol (CBN) but not cannabidiol (CBD) was shown to exhibit analgesic activity in vivo (7).

It is possible that the antiinflammatory and antiasthmatic properties of this herb are mediated through effects on arachidonate metabolism. However, constituents of *Cannabis* are known to stimulate (8, 9) and inhibit (10–12) prostaglandin (PG) release by influencing enzymes of this pathway (13, 14).

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A cannabinoid or an extract of *Cannabis* with little or no central effects could be of use therapeutically. In this paper, we have examined the antiinflammatory potential of two extracts of *Cannabis*, pure cannabinoids and olivetol (a cannabinoid biosynthetic precursor) in two models of inflammation, in an attempt to separate on a structural basis the peripheral from the central action of these phenolic drugs.

MATERIALS AND METHODS

The following were used: aspirin (Sigma Chemical Co., Poole, Dorset.), tripotassium citrate (analytical grade), all cannnabinoids except CBG (Sigma), and CBG (Makor Chemicals, Jerusalem, Israel).

Preparation of Drugs: PBQ Test. Cannabinoids and cannabis extracts were suspended in a 1% ethanolic solution containing 2.5% w/v Tween. Aspirin was dissolved in a 40 mg/ml solution of tripotassium citrate.

Phenyl Benzoquinone Writhing (PBQ) and Preparation of PBQ Solution. A 0.04% solution of PBQ was prepared immediately before use by dissolving PBQ in warm ethanol and diluting with water at 40°C (15) bringing the ethanolic concentration to 5% (16). The bottle was stoppered, foil paper wrapped around it, and the solution maintained at 34°C. Deterioration of the solution occurs if left exposed to light and air (17).

Administration of Drugs. Male CDI male (Charles River) weighing 18–20 g were starved overnight for the experiment. Animals were placed in a thermostatically controlled environment maintained at 34°C. Mice were orally administered test drug 20 min before the intraperitoneal injection of PBQ (4 mg/kg). Five minutes after injection, a hand tally counter was used to record the number of stretching movements for each mouse in a 5-min period. Control animals were only administered the vehicle. Note less than five animals were used per dose.

Statistical Analysis. Results are expressed as mean percentage inhibition of control (±*SEM*) in the case of PBQ test. IC_{50}s were obtained from graphs relating probit percentage inhibition (ordinate) against log dose (abscissa). The IC_{50} is that dose of drug which would inhibit PBQ-induced writhing by 50%.

Tetradecanoyl phorbol-acetate-induced (TPA) Erythema of Mouse Ear. In order to exclude the possibility of a central mechanism of action (see Discussion), compounds also were tested for their ability to inhibit TPA-induced erythema on mouse ears at a dose maximally effective against PBQ writhing. The lowest dose of TPA that would produce redness of ears in 100% of the animals was chosen as the challenging dose for inhibition studies, measured 4 h after application (18).

Test drugs were dissolved in ethanol and 5 μl applied to the inner ear of the mouse 15 min before the application of 1 μg TPA in 5 μl acetone. Only one dose of test drug was used for this experiment, 100 μg/5 μl ethanol, except trifluoperazine at 1 mg/5 μl. The other ear acted as a control.

The results were expressed as percentage inhibition, taken to mean the complete suppression of erythema in the test animals, as described in reference 19.

RESULTS

PBQ-Induced Writhing. CBD, CBG, olivetol, ethanolic extract, and petroleum spirit extract produced significant inhibition at doses up to 10 mg/kg

(Figures 1–3). CBN was only marginally active (Table 1). Δ^1-THC was fully effective only at concentrations above 10 mg/kg (Figure 2).

The ethanolic and petroleum extract, CBD, olivetol, CBG, and cannflavon were more potent than aspirin. The petroleum spirit extract was about four times more potent than the ethanolic extract, which was virtually equipotent with

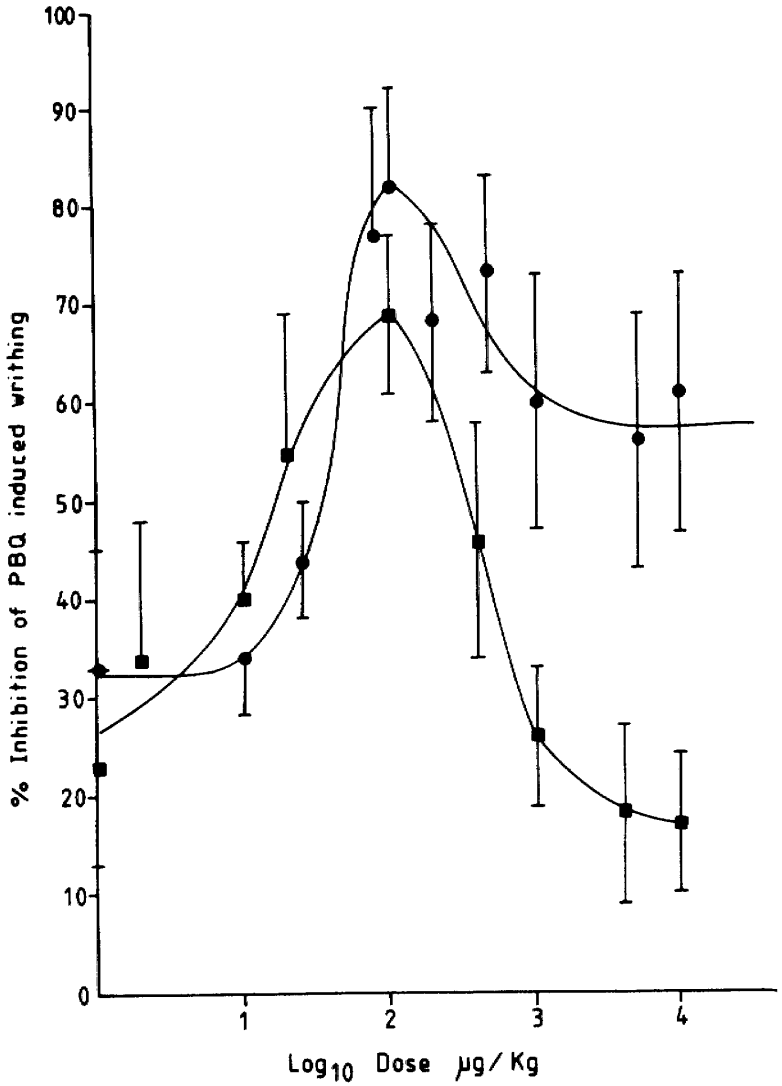


Fig. 1. Inhibition of PBQ-induced writhing (±*SEM*) by extracts of *Cannabis*. (●—●) ethanolic extract; (■—■) petroleum ether extract.

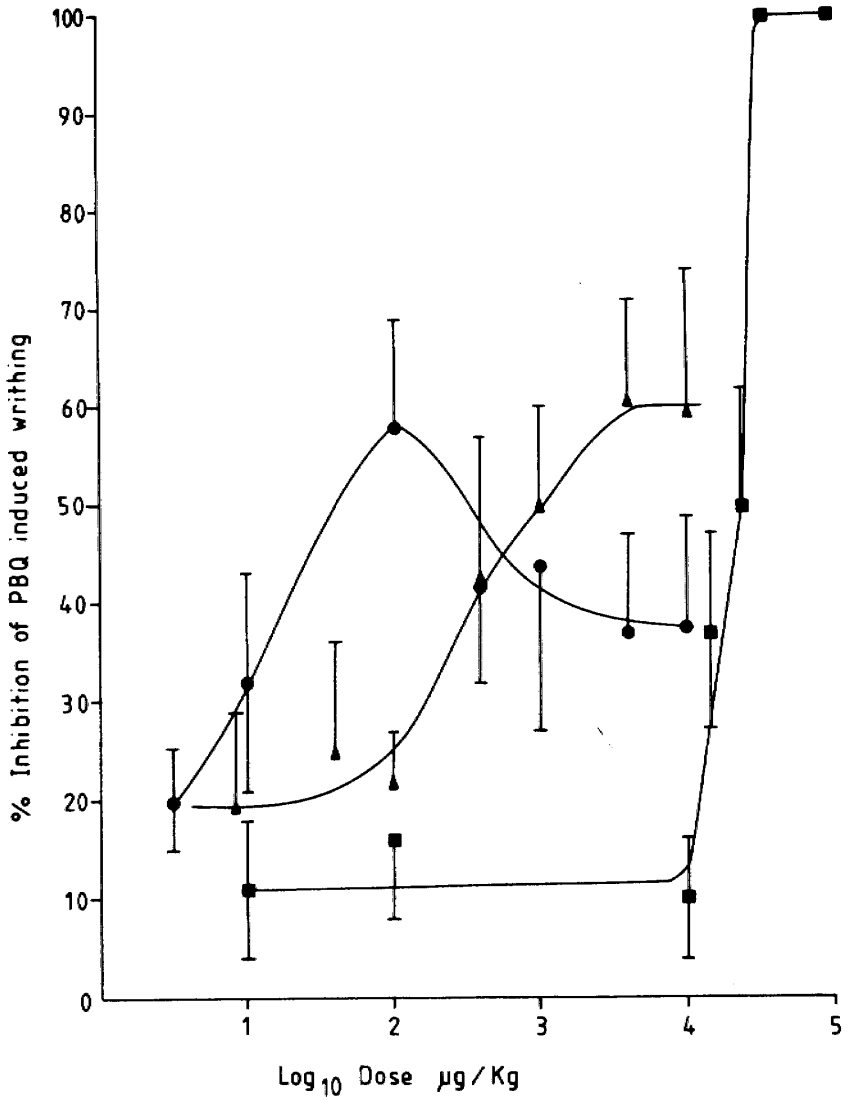


Fig. 2. Inhibition of PBQ-induced writhing by pure cannabinoids (±*SEM*) following oral administration of the drug. (●—●) CBD; (■—■) THC; (▲—▲) CBG.

CBD. Cannflavon, isolated from the ethanolic extract was 14 times less potent than the ethanolic extract of the dried herb (Table 2).

There was a decline in response following the administration of doses greater than 0.1 mg/kg of some substances. This is most evident in the bell-

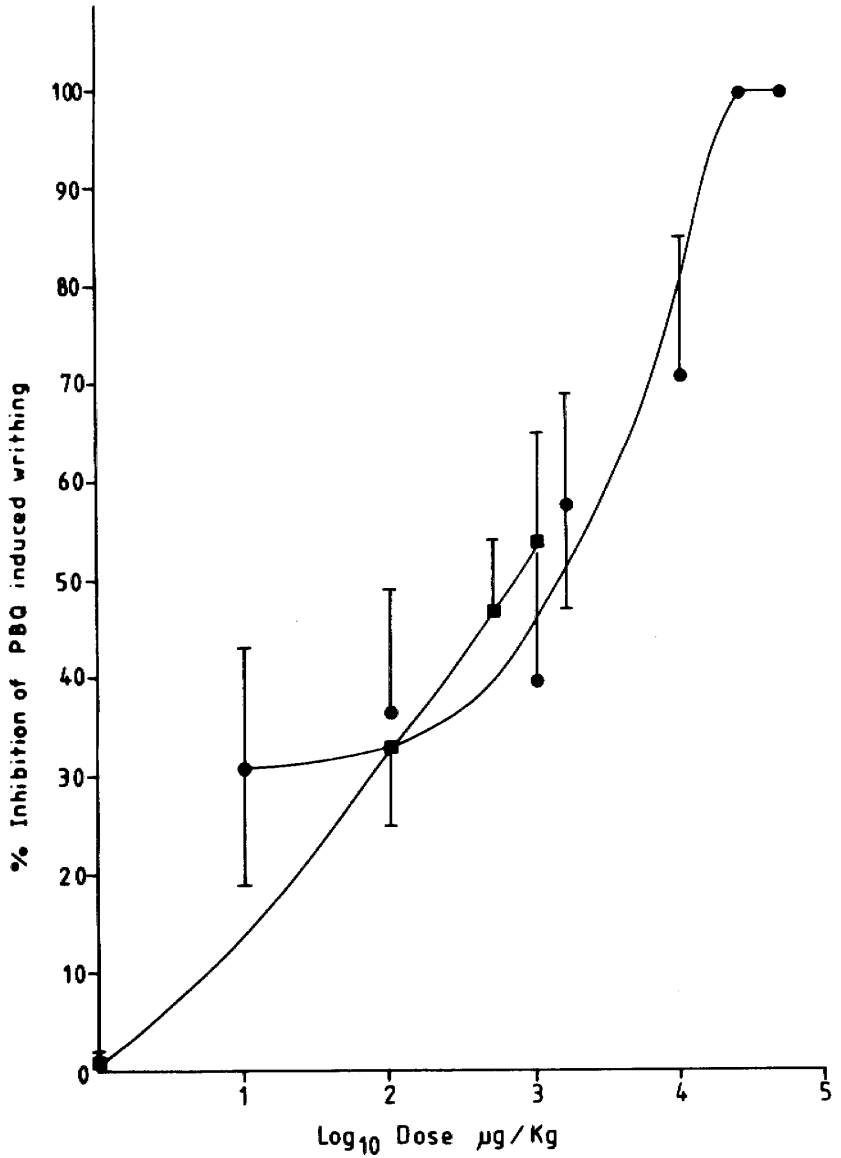


Fig. 3. Inhibition of PBQ-induced writhing (±*SEM*) by cannflavon (■—■); olivetol (●—●).

Table 1. Effect of Oral Administration of Cannabinol on PBQ-Induced Writhing in Mouse[a]

Dose (mg/kg)	Inhibition of PBQ-induced writhing (%)
0.001	31.3 ± 8.0
0.01	27.1 ± 8.0
0.1	36.5 ± 6.0
1.0	27.0 ± 6.0
10	26.0 ± 9.0

[a] Results are the mean ±*SEM*.

shaped dose–response curve of the petroleum spirit extract (Figure 1). The activity of the ethanolic extract and CBD was also found to decrease slightly at higher dose levels. (Figures 1 and 2)

TPA-Induced Erythema. In general, the ability of compounds to inhibit TPA-induced erythema correlated well with their potency in the PBQ-writhing test. Thus, CBN and Δ^1-THC were the least active followed by CBG, CBD, and cannflavon. Again, the extacts were the most active (Table 3). Twenty-four hours after application, the ethanolic extract still produced 16% inhibition of TPA-induced erythema of the animals. All other substances were without activity after 24 h.

All substances were more active than trifluoperazine, 1 mg/5 μl, a known phorbol ester antagonist both in vivo (19) and in vitro (20).

Table 2. Inhibition of PBQ-Induced Writhing in Mice[a]

Drug	Maximum conc. tested (mg/kg)	Maximum inhibition (±SEM)	Inhibition of PBQ-induced writhing, IC_{50} (mg/kg)
Aspirin	200	62.8 ± 15	15.85
Petroleum extract	10	69.8 ± 8	0.013
Ethanolic extract	10	81.8 ± 11	0.045
Δ^1-THC	50	100	25.0
CBN	10	36.5 ± 6	>25.0
CBD	10	58.0 ± 11	0.042
CBG	10	61.0 ± 10	1.26
Olivetol	25	100	0.63
Cannflavon[b]	1.0	53.9 ± 7	0.63

[a] Potency expressed as IC_{50} (the dose required to give 50% inhibition) after oral administration of drug. Efficacy expressed as maximum inhibition of the drug.
[b] Maximum concentration tested 1.0 mg/kg because of very limited quantity of the drug.

Table 3. Structure of Constituents of *Cannabis* and Percent Inhibition of TPA Induced Erythema of Mouse Ear[a]

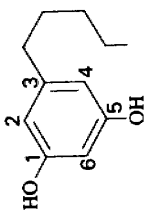
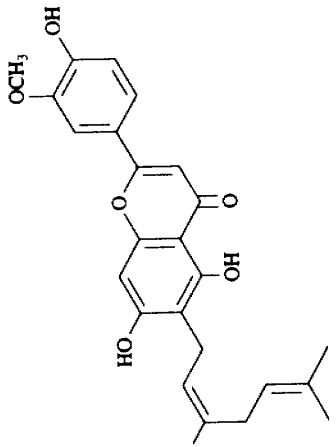
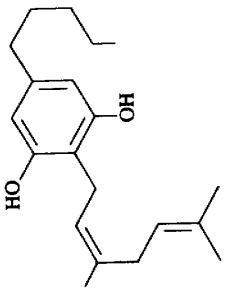
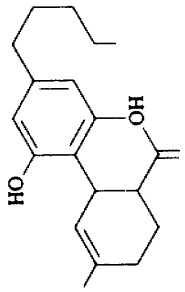
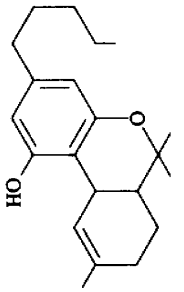
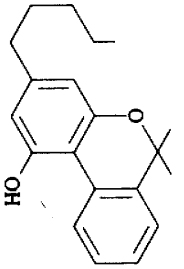
Drug	Drug Structure	Inhibition of erythema (%)
Trifluoperazine	—	50 (6)
Petroleum extract	—	83 (12)
Ethanol extract	—	92 (12)
Olivetol		62 (13)
Cannflavon		82 (11)
Cannabigerol		33 (11)

Table 3. (*Continued*)

Drug	Drug Structure	Inhibition of erythema (%)
Cannabidiol		92 (12)
Tetrahydrocannabinol		10 (12)
Cannabinol		25 (13)

[a]Number of animals given in parenthesis. All substances administered 100 μg/5 μl/ear except trifluoperazine, 1 mg/5 μl/ear.

DISCUSSION

The PBQ-induced writhing response is believed to be produced by the liberation of endogenous substance(s), notably metabolites of the arachidonic cascade (21, 22). However, the PBQ test is not specific for weak analgesics such as the nonsteroidal antiinflammatory drugs, as it also detects centrally active analgesics (16, 17). Therefore, in the elucidation of the action of the cannabinoids as inflammatory drugs, it was necessary to perform more than one test. In this case, peripheral rather than central action was confirmed in the mouse ear erythema assay.

TPA-induced erythema was inhibited by the extracts cannflavon, cannabinoids, and olivetol. The activity of TPA has been shown to be dependent upon PG release in mouse epidermis (23) and mouse peritoneal macrophages (24) possibly via the initial stimulation of protein kinase C (for a review see reference 25). It has also been shown that compounds that show moderate to very potent antiinflammatory potential in standard in vivo inflammation models will also inhibit TPA-induced edema of the mouse ear (26), and phorbol-ester-induced erythema (19).

It is possible that the cannabinoids and their extracts are inhibiting both PBQ-induced writhing and TPA-induced erythema by effects on arachidonate release and metabolism. Cannabinoids and olivetol have been shown to inhibit PG mobilization (11, 12) and synthesis (14). The noncannabinoid constituents of *Cannabis*, for example, cannflavon, have been shown to be mainly cyclooxygenase inhibitors (14). Cannabinoids, however, stimulate and inhibit phospholipase A_2 (PLA_2) activity (13), as well as inducing an inhibition of cyclooxygenase and lipoxygenase (14). The activity of *Cannabis* herb or resin is complex, in that activities can be demonstrated on at least three major enzymes of the arachidonate cascade.

The mechanism by which Δ^1-THC inhibits PBQ-induced writhing may differ from that of the other substances. At concentrations greater than 10 mg/kg, Δ^1-THC may be inhibiting PBQ-induced writhing by acting on central rather than peripheral functions. It is possible that prostaglandins modulate certain inhibitory pathways in the brain, bringing about an increase in the pain threshold. This dose of Δ^1-THC is capable of bringing about the cataleptic effect (27), which is a standard test for central involvement. Central analgesics have higher efficacies than peripheral ones, and this may explain the effectiveness of Δ^1-THC (Figure 2). The central involvement of Δ^1-THC is perhaps the primary reason why Δ^1-THC was recognized as an analgesic before other cannabinoids.

Our results suggest that the response of the ethanolic extract cannot be solely due to cannflavon. Other structurally related phenolic substances, known to be present in this complex extract, may account for the higher activity seen

either due to cumulative or synergistic effects upon cyclooxygenase. The activity of the petroleum ether extract is likely to be largely due to the presence of CBD and CBN. GLC analysis of the extract has shown that this extract contained 14.13% CBD, 9.08% CBN, and 6.68% Δ^1-THC (27). On the basis of our results, it is possible to separate the centrally active cannabinoid Δ^1-THC from peripherally active compounds of the herbal extracts. An attempt has been made to differentiate them structurally (Table 3). It can be seen that the olivetolic nucleus together with a free C-5 hydroxyl group are structural requirements for peripheral effects, involving both cyclooxygenase and lipoxygenase inhibition (14). Substances possessing this structure possess antiinflammatory and analgesic activities without central hallucinogenic effects. Δ^1-THC and CBN, which are cyclized derivatives exhibiting no C-5 hydroxyl moiety, have little if any peripheral action. The traditional use of *Cannabis* as an analgesic, antiasthmatic, and antirheumatic drug is well established. Our results would suggest that cultivation of *Cannabis* plants rich in CBD and other phenolic substances would be useful not only as fiber-producing plants but also for medicinal purposes in the treatment of certain inflammatory disorders.

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