# The pharmacological effect of fractions obtained by smoking cannabis through a water-pipe. II. A second fractionation step

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*Summary.* The catatonic activity, prolongation of phenobarbital sleeping-time, convulsant action and disruption of nest-building activity were assessed in mice subjected to 4 cannabis pyrolysis products and their tobacco analogues. All but one of the cannabis fractions prolonged the pentobarbital sleeping-time and disrupted the nest-building activity of mice in a way not related to their content in the main cannabinoids. Nest-building activity seems to be the most valid assay we have used so far.

Previous work in this and other laboratories has shown that 2 of the fractions obtained by smoking cannabis through a water-pipe possess cannabis-like activity in several biological assays[1-3]. The crucial point of these papers has been the relative independence between the effects of the cannabis products and the dose of $\Delta^9$-THC administered with them. 2 hypotheses were put forward to explain this finding: a) other main cannabinoids (like CBD) may interfere with the $\Delta^9$-THC activity[4-6], and b) low volatile tobacco principles retained in the water-phase may modify the activity of $\Delta^9$-THC[1].

The aim of this paper is to test the validity of these 2 hypotheses. Therefore, a) we tried to see whether CBD would interfere with $\Delta^9$-THC in one of the biological assays we used in the past, and b) we proceeded one step farther with the fractionation of cannabis pyrolysis products in order to gather more fractions differing at least in their concentration in cannabinoids. We report here the analytical and pharmacological work on these fractions.

*Material and methods.* 2 active cannabis products were gathered, as in a previous experiment, after smoking cannabis (United Nations' reference Cannabis UNC 351) through a water-pipe. These were: 1. $III_s$, the particulate material of the smoke that usually enters the lungs of hashish-smokers, and 2. $II_s$, the non-soluble and non-volatile substances that remain on top of the water or on the walls of the water-container of the pipe used.

These 2 fractions were extracted several times with petroleum ether(PE). 4 fractions (A–D) were thus obtained and analyzed by means of TLC and gas chromatography for the main cannabinoids, $\Delta^9$-THC, CBD and CBN:

A = substances removed with PE (SPE) from fraction $III_s$: $\Delta^9$-THC: 7.18%, CBD: 3.29% and CBN: 25.28%.

B = substances contained in $III_s$ that remained after extraction with PE (SnPE): $\Delta^9$-THC: 3.32%, CBD: 1.32% and CBN: 4%.

C = substances removed with PE (ZPE) from fraction $II_s$: $\Delta^9$-THC: 4.77%, CBD: 1.98% and CBN: 15%.

D = substances contained in $II_s$ that remained after extraction with PE (ZnPE): $\Delta^9$-THC: 3.50%, CBD: 1.98% and CBN: 15%.

Analogous extraction of the tobacco products $II_B$ and $III_B$ gave another 4 fractions ($SPE_B$, $SnPE_B$, $ZPE_B$ and $ZnPE_B$), which were naturally devoid of any cannabinoids. The w/w ratio between PE and nPE fractions was 4:6. We adjusted the doses we administered according to this ratio.

The pharmacological activity of these fractions was assessed in mice as far as the following were concerned: prolongation of pentobarbital sleeping time, catatonic activity, synergism with phenytoin in protection from electrically induced convulsions, and interference with nest-building behavior. The drugs were injected i.p. (injection volume 10 ml/kg) in the form of a suspension in Tween-80 and saline (1:99 v/v). We used more than 400 male albino mice, 3–5 months old, in the 1st 3 pharmacological assays and 30 $C_{57}$Bl male mice in the behavioral one(nest-building). The animals were kept in an animal house under 12 h light-12 h dark illumination and a constant temperature of 23 °C.

I. Prolongation of pentobarbital sleeping time: We followed the procedure described by Savaki et al.[1]. The animals were first injected with the control solution (saline and Tween-80) or one of the fractions under study. 1 h later the animals were injected with 50 mg/kg of pentobarbital sodium. 3 comparisons were carried out by means of the Mann-Whitney U-test: 1) Control solution, 4 mg/kg of the fractions SPE, $SPE_B$, ZPE, $ZPE_B$ and 6 mg/kg of the fractions SnPE, $SnPE_B$, ZnPE, $ZnPE_B$. 2) Control solution, 16 mg/kg of the fractions SPE, $SPE_B$, ZPE, $ZPE_B$ and 24 mg/kg of the fractions SnPE, $SnPE_B$, ZnPE, $ZnPE_B$. 3) Control solution, 50 mg/kg of CBD, 32 mg/kg of SPE, $SPE_B$, ZPE, $ZPE_B$, and 48 mg/kg of the fractions SnPE, $SnPE_B$, ZnPE, $ZnPE_B$.

II. Catatonic activity: We followed the procedure described by Savaki et al.[1]. The animals were injected i.p. with either 10 mg/kg of $\Delta^9$-THC, or 8 mg/kg of SPE, $SPE_B$, ZPE, $ZPE_B$ or 12 mg/kg of SnPE, $SnPE_B$, ZnPE, $ZnPE_B$.

III. Anticonvulsant activity: This was evaluated in a maximal electroshock test (MES). The shock (10 mA 60 Hz sinusoidal current of 1-sec duration) was given through corneal electrodes. The apparatus was similar to the one suggested by Woodbury and Davenport[7]. The time course of the anticonvulsant effect was determined by the use of the ratio of extensor to flexor time (E/F). A decrease in E/F ratio is indicative of anticonvulsant activity. The animal was shocked 2 h after injection with phenytoin sodium (Epanutin-Parke Davis, 1 mg/kg) and saline or $\Delta^9$-THC (40 mg/kg) or one of the pyrolysate fractions (24 mg/kg of SPE, $SPE_B$, ZPE, $ZPE_B$ and 36 mg/kg of SnPE, $SnPE_B$,

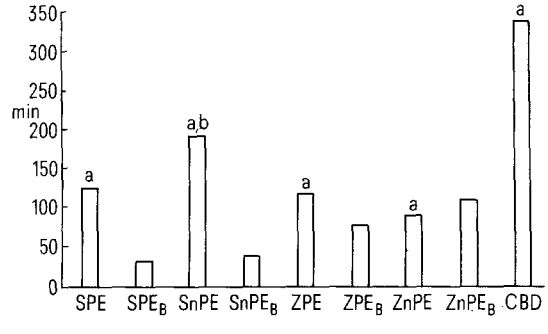


Fig. 1. Effects of tobacco fractions ($SPE_B$, $SnPE_B$, $ZPE_B$ and $ZnPE_B$) and of cannabis fractions (SPE, SnPE, ZPE and ZnPE) and of CBD on the pentobarbital sleeping-time of mice. The columns represent increase of means (in min) as compared with the control values. The control sleeping-time in the 3 comparisons were, respectively, 87.55 ± 33.73, 115.33 ± 34.99 and 95.7 ± 33.88 min (mean ± SD). *a* indicates statistically significant difference ($p < 0.01$) from the control group (Wilcoxon test). *b* indicates statistically significant difference ($p < 0.01$) from the corresponding tobacco placebo (Mann-Whitney U-test).

ZnPE, $ZnPE_B$), since this is the peak-effect time for cannabinoids suggested by many authors[8-11]. We kept the ambient temperature at 20 °C during the experiment.

IV. Interference with nest-building behavior: We followed the procedure described by Moschovakis et al.[2]. The duration of the retrieval of the fresh nesting material to the corner chosen as nest site served as an index of the mice's performance. The animals were injected with doses of 24 mg/kg for SPE, $SPE_B$, ZPE, $ZPE_B$, 36 mg/kg for SnPE, $SnPE_B$, ZnPE, $ZnPE_B$ and 5 mg/kg of $\Delta^9$-THC. In a further effort to explain the discrepancy between $\Delta^9$-THC content in our fractions and magnitude of the behavioral effect, we examined the interaction between $\Delta^9$-THC and CBD in the same biological assay. Thus we injected the animals with 10 mg/kg $\Delta^9$-THC or 10 mg/kg CBD or a combination of the above doses of the same drugs. Comparison between test and saline-control injections was carried out with the Wilcoxon non-parametric test, whereas comparison between experimental and tobacco-control fractions was carried out with the Mann-Whitney U-test.

*Results and discussion.* Only the high doses of the experimental fractions significantly prolonged the pentobarbital-induced sleeping time. SnPE was the most potent fraction in this respect ($p < 0.025$) (figure 1). This is not suprising since SnPE is relatively more abundant in CBD than any other fraction. CBD alone was far more active than any other drug ($p < 0.001$). The large SD in the data gathered should be noted (half the order of magnitude of the mean) and that it confirms the results of Savaki et al.[1] 'Active substances from tobacco, probably nicotine, which possess both stimulant and depressant actions' may indeed be responsible for the somewhat different performances of different animals.

No fraction was found to induce catatonia in mice (the 10 mg/kg of $\Delta^9$-THC induced a catatonia that lasted for more than 1 h). This is not the first time cannabis products have been found to be devoid of any catatonic activity[1]. Thus we do not feel Loewe's classical assay should be used anymore in cannabis psychopharmacology, at least in experiments where small doses of cannabinoids are being used.

SPE was the only fraction significantly more potent in protecting mice from electrically induced convulsions in comparison to its tobacco control ($p < 0.025$, Mann-Whitney U-test). However, as the E/F ratio ranged between 4 and 6 for the cannabis products and between 5.5 and 6.5 for their tobacco controls, we feel further experiments are necessary if the anticonvulsant activity of tobacco is to be ruled out (Saline control E/F ratio: 10.375).

5 mg/kg of $\Delta^9$-THC and SPE, SnPE, ZPE in the doses administered, similarly disrupted the nest-building activity of mice both qualitatively and quantitatively (figure 2). It is not likely that this is due only to the presence of $\Delta^9$-THC since its dose contained in these 3 fractions did not exceed 1.8 mg/kg, 0.8 mg/kg and 1.7 mg/kg, respectively. Many authors have stressed the role of other known cannabinoids in defining the activity of cannabis products. We do not think this is the case as far as interference with nest-building behavior is concerned, because at least CBD has no effect of its own and does not seem to modify the activity of $\Delta^9$-THC (figure 3).

Others have stressed the importance of tobacco non-volatile substances in this unexpected potentiation of the $\Delta^9$-THC activity of cannabis pyrolysis products. We do not feel the characterization non-volatile is well substantiated. Whatever its chemical basis, this potentiation is evenly distributed in almost every cannabis pyrolysis product we have tested until now. Thus we feel that further fractionation steps are necessary if the substance(s) responsible are to be revealed. In conclusion, we would like to emphasize the usefulness of the nest-building model in this respect, since its simplicity matches well with the clearcut, all-or-nothing profiles it provides for experimenters working in the field.

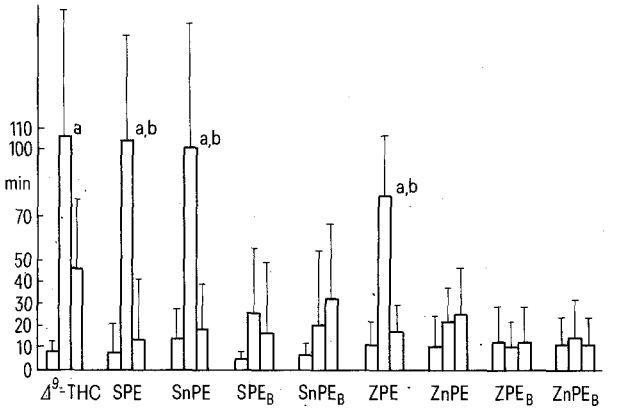


Fig. 2. Prolongation of retrieval phase in mice nest-building behavior after injecting several drugs. Column 1: saline control before test; column 2: test; column 3: control after test. *a* indicates a statistically significant difference ($p < 0.01$) from predrug saline injection (Wilcoxon test). *b* indicates statistically significant difference ($p < 0.01$) from the corresponding tobacco placebo (Mann-Whitney U-test).

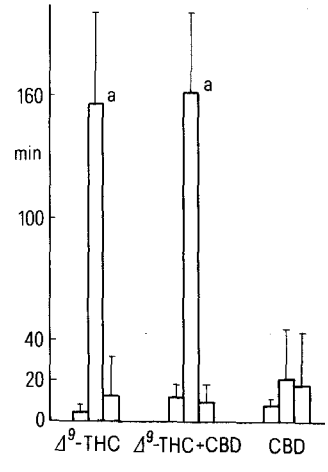


Fig. 3. Prolongation of retrieval phase in mice nest-building behavior after injection of $\Delta^9$-THC, CBD and a combination of $\Delta^9$-THC and CBD. The columns for every drug are grouped as in figure 2.

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