Marihuana Components: Effects of Smoking on Δ^9-Tetrahydrocannabinol and Cannabidiol

Abstract. *Cigarettes impregnated with various preparations of Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol were artificially smoked, and the smoke was analyzed by means of gas chromatography and a combination of gas chromatography and mass spectrometry. Retention of the two test substances in smoke was 21 to 23 percent of the starting amount. The ratio of Δ^9-THC to cannabidiol in smoke is different from that in starting material. It is suggested that these differences are the result of partial cyclization of the cannabidiol to Δ^9-THC. An increase in the percentage of cannabinol in smoke is the result of a partial dehydrogenation of Δ^9-THC or cannabidiol. No evidence for the isomerization of Δ^9-THC to Δ^8-THC or for the formation of new pyrolyzed products was found.*

Hashish is two to three times more active when smoked than when taken orally. The same holds for *trans*-10,6a (—)-Δ^9-tetrahydrocannabinol (Δ^9THC) which is only approximately 95 percent pure (*1*). One possible explanation is that during combustion some of the inactive components of marihuana are converted to active components. We have studied the effects of smoking on psychotomimetically active Δ^9THC and inactive cannabidiol (CBD) (two main constituents of marihuana) (*2*, *3*) to determine to what degree these compounds are affected by oxidation and which, if any, new active products are produced.

Table 1. Gas chromatographic analysis of marihuana constituents and resin before smoking; + indicates trace.

Material	Percentage			
	Δ^9THC	CBD	CBN	Unidentified
Synthetic Δ^9THC	95	4	0.05	1.0
Synthetic CBD	3	96.3	0.7	+
Marihuana resin	0.7	0.6	1.5	+

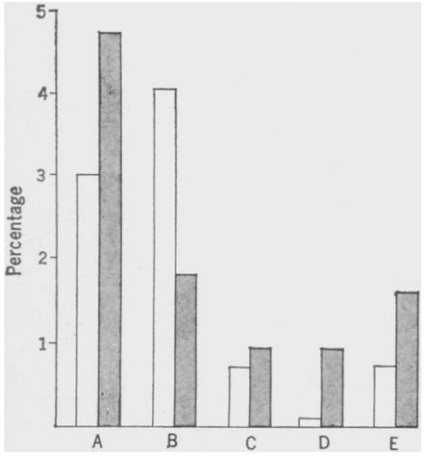

Fig. 1. Concentrations of marihuana components: (open bars) concentrations before smoking; (shaded bars) concentrations after smoking. Standard deviation, 3.6 percent; (*A*) Δ^9THC in CBD; (*B*) CBD in Δ^9THC; (*C*) Δ^9THC in marihuana; (*D*) CBN in Δ^9THC; (*E*) CBN in CBD.

Using gas chromatography and gas chromatography coupled with mass spectrometry we determined the contents of our starting material (Table 1). Then the test substances were dissolved in ether. One milliliter of the solution containing 950 μg of Δ^9THC, 963 μg of CBD, or 20.2 mg of marihuana resin was injected into the middle third of a Pall Mall cigarette (without filter, 8.4 cm long). The cigarettes were connected to filters (Cambridge) and then "smoked" by means of either a smoking machine (Filtrone/I, Cigarette Components Ltd., London) or a 50-ml suction syringe. Experiments were carried out so that 35 ml of smoke was "inhaled" in 2 to 3 seconds. Cigarettes were smoked for no longer than 7 minutes by machine or between 3 to 6 minutes by syringe. The number of "inhalations" varied from 15 to 25, and cigarettes were smoked to leave a butt of 17 mm. After smoking, the filters of 14 cigarettes, including two untreated controls, were soaked three times in 15 ml of ether, first for 20 minutes and then twice for 3 minutes. The different extracts were pooled, evaporated at room temperature to 1 ml, and then analyzed by gas chromatography or combined gas chromatography and mass spectrometry (Table 2). The two methods of artificially smoking cigarettes yielded the same results (*5*).

The amount of unpyrolized components (Δ^9THC and CBD) in the smoke acquired from the machine or syringe ranged between 21 and 23 percent. This finding is in agreement with data from studies on tobacco alkaloids (nicotine and so forth), which also are combusted approximately 80 percent during smoking. No Δ^8THC isomers were formed when cigarettes impregnated with the synthetic Δ^9THC were smoked. This result supports the prevalent idea that isomerization of Δ^9THC to Δ^8THC does not occur (*3*, *6*). Results of combined gas chromatography and mass spectrometry indicated no new pyrolytic products (*7*) when mass spectra from control cigarettes and those treated with Δ^9THC were compared (for gas chromatography the temperature was programmed to increase 3°C/min, from 100° to 230°C).

Extracts from smoked cigarettes impregnated with solutions of synthetic CBD and natural marihuana resin were also analyzed by combined gas chromatography and mass spectrometry. CBD and Δ^9THC were identified by their retention times ($t_r = 2.9$ and 3.6 minutes, respectively) and by typical fragments ($M^+ = 314$, $M^+ - CH_3 = 299$, $M^+ - C_6H_{11} = 231$) before and after smoking.

When we compared chromatographic data for the starting material and the extracts after smoking (Fig. 1), we observed a tendency for the percentage of Δ^9THC to increase as a result of smoking when a much higher concentration of CBD than Δ^9THC is present initially. From these findings it is possible to hypothesize that a small part of the CBD is being cyclized into Δ^9THC during smoking (Fig. 2.) This process could occur at acidic *p*H (as in cigarette smoke) through ring closing in CBD (*8*). This could further explain the observed activity of marihuana with a low amount of Δ^9THC

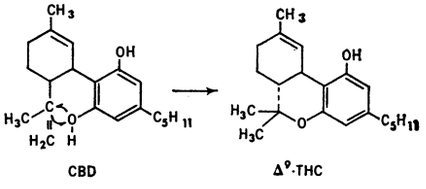

Fig. 2. Conversion of CBD to Δ^9THC.

Table 2. Gas chromatographic analysis of two preparations of Δ^9THC and CBD used in combustion studies.

Component	Percentage	
	Before smoking	After smoking
	Preparation 1	
Δ^9THC	95	22.5
CBD	4	1.8
	Preparation 2	
CBD	96.3	21.2
Δ^9THC	3	4.7

in human smokers. Additional enrichment of Δ^9THC and Δ^8THC may come from decarboxylation of Δ^9THC and Δ^8THC acids to their active THC isomers, which also is known to take place (*9*). Experiments with labeled substances should provide further evidence for partial cyclization of CBD. Finally, the observation that there is a relative increase of cannabinol (CBN) in either synthetic CBD or synthetic Δ^9THC with smoking (Fig. 1) may be accounted for by dehydrogenation of these substances to CBN.

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References and Notes

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2. H. Aramaki, N. Tomiasu, H. Yoshimura, H. Tsukamoto, *Chem. Pharm. Bull. (Japan)* **14**, 262 (1968).
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4. Gas chromatography was performed with a Fractovap GI-450 gas chromatograph (Carlo Erba, Milan) on a column (glass spiral, 2.7 m long with internal diameter of 3 mm) filled with 1 percent SE-30 on Chromosorb W (silanized). Nitrogen was used as carrier gas (30 ml/min), and the column temperature was 225°C. A flame ionization detector was used. For combination gas chromatography and mass spectrometry, an LKB model 9000 combination gas chromatograph and mass spectrometer (LKB, Stockholm) was used. Conditions for gas chromatography remained as above except that helium was used as a carrier gas, and a total ion current detector was used. Of the gas chromatograph effluent, 20 percent goes to the detector and 80 percent goes to the mass spectrometer. Conditions for mass spectrometry were: ionization potential, 70 ev; ion and separator temperatures, 270°C; injector temperature, 250°C; multiplier voltage, 3.5 kv; trap current, 65 μa.
5. Average of five cigarette test samples, eluted in ether. Two further cigarette filters eluted in 20 ml of cooled alcohol showed only traces of the smoked substances.
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7. D. H. Efron, Ed., *Psychotomimetic Drugs* (Raven, New York, 1970), p. 319.
8. One of the experimental syntheses of Δ^9THC through cyclization of CBD was done under acidic conditions as described by Y. Gaoni and R. Mechoulam [*J. Amer. Chem. Soc.* **86**, 1646 (1964)] and R. Mechoulam and Y. Gaoni [*ibid.* **87**, 3273 (1965)]. When CBD was artificially heated to 300°C for 48 hours in a stream of oxygen or treated with acid, it was converted to tetrahydrocannabinols as described in *Hashish: Its Chemistry and Pharmacology*, C. E. W. Wolstenholme and J. Knight, Eds. (Churchill, London, 1965).
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10. We thank Dr. D. H. Efron at the National Institute of Mental Health, Bethesda, Maryland, for his helpful discussion and corrections of the manuscript; Dr. T. Petrzilka at the Swiss Federal Institute of Technology, Zürich, for synthetic Δ^9THC and CBD; Dr. A. Chang Sin Ren for a translation from Japanese; and G. Boshart and N. Waser for their assistance with the English translation.

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Histochemical Fluorescence of Raphe Neurons: Selective Enhancement by Tryptophan

Abstract. *The histochemical fluorescence of those neurons in brainstem raphe nuclei which are presumed to contain serotonin is selectively and stereospecifically enhanced by* L-*tryptophan at doses that also produce an elevation in the concentration of serotonin. However, contrary to our assumptions, the increase in raphe fluorescence is not prevented by* p-*chlorophenylalanine, an inhibitor of serotonin synthesis. These results suggest that under some conditions derivatives of tryptophan other than, or in addition to, serotonin may be of significance in raphe neurons.*

Neurons whose perikarya are situated in the raphe nuclei of the brainstem have been implicated in a variety of functions including slow-wave sleep (*1*), habituation to sensory stimuli (*2*), and the action of D-lysergic acid diethylamide (*3*). Evidence derived from histochemical fluorescence (*4*) and other methods (*5*) suggests that raphe cells and their processes comprise the principal, if not the only, neurons in the brain containing serotonin [5-hydroxytryptamine (5-HT)]. However, existing approaches do not provide definitive proof of this thesis, as is illustrated by the fact that the histochemical fluorescence studies employ drugs that are not entirely specific for 5-HT (for example, monoamine oxidase inhibitors and reserpine) (*4*). Furthermore, loading doses of the immediate precursor of 5-HT, 5-hydroxytryptophan, produce a large increase in the concentration of 5-HT in brain (*6*), but fail to enhance appreciably the fluorescence of raphe neurons except in the presence of other drugs (*4, 7*). This negative result is possibly accounted for by the nonspecific decarboxylation of 5-hydroxytryptophan and subsequent accumulation of 5-HT in abnormal sites [for example, endothelial cells (*4, 7*)].

Loading doses of tryptophan also cause an elevation in the concentration of 5-HT in the brain (*8*), presumably because tryptophan hydroxylase, the enzyme involved in the initial step of 5-HT synthesis, has a high Michaelis constant (K_m) (3×10^{-4} mole/liter), and concentration of tryptophan in the brain would not ordinarily saturate the enzyme (*9*). However, tryptophan hydroxylase, unlike enzymes that decarboxylate 5-hydroxytryptophan, should be selectively localized within cells normally containing 5-HT. Consistent with this notion is the fact that the regional distribution of 5-HT follows a normal pattern after loading with tryptophan but not 5-hydroxytryptophan (*10*). On this basis, we reasoned that loading doses of tryptophan would be effective in increasing the intensity of fluorescence of raphe neurons.

The methods we used for the histochemical fluorescence experiments involved modifications (*4*, *11*) of the formaldehyde condensation procedure for demonstrating monoamines in freeze-dried tissue as originally described by Falck *et al.* (*12*). In brief, a total of 60 control or treated Charles River (C.D.) male rats were guillotined, and their brains were rapidly removed. Coronal slices, approximately 1 mm thick, were cut and rapidly quenched in a mixture of propane and propylene (10:1) cooled by liquid nitrogen. The tissues were then placed in a desiccator over P_2O_5 and freeze-dried in a vacuum at −45°C for 1 week. The dried tissue was exposed (1.5 hours) to formaldehyde gas generated at 80°C from paraformaldehyde powder (1 g per 100 ml of chamber). The paraformaldehyde had previously been equilibrated with an atmosphere of 60 percent relative humidity. Tissues were embedded in paraffin in a vacuum, and sections (15 μm thick) were cut and mounted on glass slides in nonfluorescent emersion oil. Slides were examined by means of

Table 1. Effect of L-tryptophan loading on concentrations of serotonin (5-HT) in whole brain of control rats and those previously treated with *p*-chlorophenylalanine. Rats injected with DL-*p*-chlorophenylalanine methyl ester hydrochloride (PCPA) received 400 mg/kg intraperitoneally. Those rats treated with both PCPA and L-tryptophan received an injection of PCPA 28 hours before intraperitoneal injection of L-tryptophan (100 mg/kg) or vehicle (10 ml/kg). L-Tryptophan was dissolved in a vehicle (10 mg/ml) consisting of one drop of Tween per milliliter of distilled water. The changes from control are all significant at $P < .001$.

Treatment	Animals (No.)	5-HT (ng/g ± S.E.)	Change from control (ng/g)
Control	9	340 ± 13	
PCPA	4	67 ± 13	− 273
L-Tryptophan	7	496 ± 14	+ 156
PCPA + L-tryptophan	7	112 ± 12	− 228