Fluorescence of cannabinoids

The formation of fluorescent derivatives of cannabinoids has been reported recently (Forrest, Green & others, 1971) and their use as a potential means of cannabinoid detection in biological fluids has been described (Just, Lerner, & Wiechmann, 1972). We wish to report that cannabinoid fluorescence may be induced by thermal treatment of cannabinoids and we offer evidence in support of the use of this property for the identification of cannabis constituents in human urine.

Thus, 1 mg of Δ^9-tetrahydrocannabinol (Δ^9-THC) was heated in the solid state for 15 min at 230° and after it had cooled to room temperature, it was dissolved in 1 ml of n-heptane and the solution filtered. The filtrate on examination by spectrofluorimetry (Aminco-Bowman spectrophotofluorometer) gave the fluorescence characteristics: λ_{max} excitation 370 nm, λ_{max} emission 420 nm (Fig. 1.) In the emission spectrum, the 370 nm shoulder is attributable to reflections from the excitation beam and the 440 nm shoulder occurs in the thermal products of several different samples of Δ^9-THC. The THC was isolated by column chromatography from extracts of cannabis resin and was purified by repeated preparative t.l.c. on Kieselgel-G plates. To obtain blank values, a substance-free zone of Kieselgel-G was eluted with chloroform and treated as above for THC. The relative fluorescence intensity of these materials was unimportant.

Dilutions of the solution with n-heptane followed by measurement of the fluorescence of the diluted solutions, indicated that measurable fluorescence intensity could be obtained with thermal treatment product corresponding to not less than 0·1 μg of Δ^9-THC.

Similar results were obtained by heating cannabidiol (CBD) or cannabinol (CBN), though it was found that, in both cases, the λ_{max} emission was moved slightly towards higher wavelengths (440 nm).

The thermally obtained products were compared with starting material using t.l.c. which indicated that the heated THC was decomposed or otherwise modified into seven spots separated by t.l.c. analysis (reacting with Fast Blue B.B. salt).

It has been reported that, though in negligible amounts, Δ^9-THC is excreted as such in the urine, after its administration to man (Miras & Coutselinis, 1970). It has, also, been found in this laboratory that unchanged CBN is excreted in the urine of chronic cannabis smokers (Miras & Dionyssiou-Asteriou, unpublished results). It was

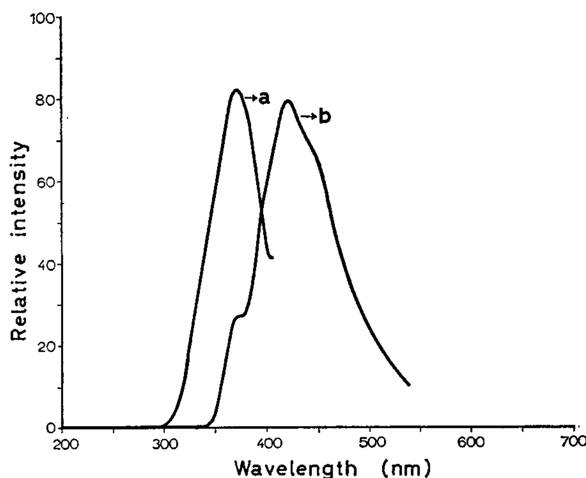

Table 1. *Effect of the increase in the amounts of the main cannabinoids, taken by smoking, on the fluorescence produced by urine extracts.* One regular cannabis smoker was used. Each cannabis sample (containing the indicated amounts of Δ^9-THC, CBD and CBN) was smoked by him twice a day for two consecutive days. Urine samples were collected in the morning of the third day (in each case). 75 ml from each sample was treated as described in the text. The residue was heated at 230° for 15 min and after cooling to room temperature it was dissolved in 1 ml of n-heptane, the solution filtered and the fluorescence of the filtrate measured as described for Δ^9-THC.

Cannabis sample	Amount (in mg) contained in the cannabis sample			Relative fluorescence intensity (per mg) creatinine $\times 10^{-2}$)
	THC	CBD	CBN	
I	7	6	2	0·77
II	16	14	5	2·10
III	20	18	6	2·60

thought reasonable, therefore, to investigate the possibility of obtaining a fluorescence picture, from the urine of cannabis users, analogous to that obtained from Δ^9-THC, CBD and CBN as described above.

Thus, 6 regular cannabis users were subjected to smoking 200 mg of cannabis in the evening and their first morning urine collected separately. Seventy five ml of urine from each individual were acidified to pH 3·4 and extracted with an equal volume of light petroleum (40°—60°). The organic layer was collected, the solvent removed under reduced pressure, and the residue thermally treated as described above for Δ^9-THC. The light petroleum extracts gave essentially the same fluorescence spectra as the thermally-treated original THC.

The same procedure was followed for urine specimens taken from fifty individuals used as controls. Most of these controls were regular tobacco smokers. (The fluorescence spectra of the urine from tobacco smokers did not show any interfering peaks.)

Relative fluorescence intensities obtained from both groups are: cannabis smokers (6) 1·28 $\pm$ 0·21, non-smokers of cannabis (50) 0·23 $\pm$ 0·025 (means $\pm$ s.e.). from which it is clear that cannabis smokers may be distinguished from individuals not using the drug, on the basis of the large difference in the fluorescence intensity between the two groups. That the high fluorescence intensity observed in the group of cannabis smokers is due to cannabinoid substances, present in their urine, is supported by the fact that increase in the amounts of cannabinoids taken by smoking, is followed by a similar increase in the intensity of the fluorescence as shown in Table 1.

It must be emphasized that forty of the individuals used as controls were patients hospitalized for various diseases who had been given a wide variety of drugs. The low fluorescence intensity observed in these individuals suggests that interference of commonly used drugs can be excluded. This, together with the highly significant difference in the fluorescence intensity between cannabis users and non-users supports the view that fluorescence of cannbinoids induced by their thermal treatment might be used for their identification in the urine of cannabis takers.

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Apparent enhanced stability in acid media of the ethyleniminium ion derived from dibenamine

Studies on the inhibition reaction between α-chymotrypsin and dibenamine (Al Shabibi & Smith, 1974) showed that the alkylating species were derived from dibenamine and that this species, which was probably the ethyleniminium ion, was present and stable in acid media but was rapidly depleted in near neutral buffered media. We have assessed the relative levels of this ion in solutions of dibenamine in a qualitative manner using its known blocking action on the muscarinic receptor of guinea-pig isolated ileum (Furchgott, 1954).

Materials. Strips of terminal guinea-pig ileum were suspended in 5 ml of aerated Tyrode solution at 37°. Muscle contracture was recorded isotonically under a load of 1 g by means of a simple lever and a four-fold magnification. The time cycle for emptying and refilling the tissue bath with Tyrode solution was controlled by the relays of an automatic assay command module (Cassella Electronics).

Inhibition of the muscarinic receptor by dibenamine

In all experiments the maximal response of the tissue to acetylcholine was determined before a dose giving 50% maximal response (submaximal dose) was assessed from a log dose-response curve. The contact time was 30 s. This was followed by flushing the bath with its own volume of Tyrode solution. There was a rest period of 3 min between each addition of acetylcholine. When the response to the submaximal dose of acetylcholine was reproducible, the incubation with dibenamine solution was commenced.

The tissue was incubated for 2 min with a solution of dibenamine hydrochloride ($1{\cdot}6 \times 10^{-5}$ M) freshly prepared in Tyrode solution (pH 7·4). The bath was then washed out once with Tyrode solution and the submaximal dose of acetylcholine was applied on three occasions each of 30 s with intermediate washing with Tyrode as before. The average value for the height of contracture was used in subsequent calculations. The whole procedure of incubating the tissue with dibenamine and