Two cannabidiol metabolites formed by rat liver

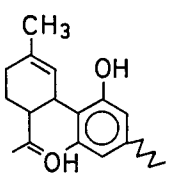
Cannabis sativa L. contains three major cannabinoids, *i.e.* Δ^1-tetrahydrocannabinol (Δ^1-THC*), cannabinol (CBN) and cannabidiol (CBD; I). It appears that only Δ^1-THC is psychotomimetically active but its effect is apparently modified by other compounds also present in Cannabis (*cf.* Mechoulam, 1970). From recent evidence (Paton & Pertwee, 1972; Jones & Pertwee, 1972) it is likely that CBD contributes to the activity of cannabis preparations by inhibiting the metabolism both of Δ^1-THC and its primary active metabolite 7-hydroxy-Δ^1-THC.†

We have now studied the metabolism of CBD using rat liver homogenate 10 000 *g* supernatant.

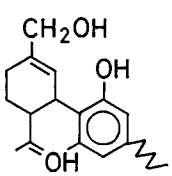
[^{14}C] CBD was prepared from [^{14}C] olivetol (Nilsson, Nilsson & Agurell, 1969) using the method of Petrzilka, Haefliger & Sikemeier (1969). The labelled CBD (30 mg) was incubated with 10 000 *g* supernatant from rat liver homogenate using an *in vitro* system described by Tagg, Yasuda & others (1967) with the modifications previously reported (Nilsson, Agurell & others, 1970).

The incubation mixture was extracted with light petroleum to remove most of the unchanged CBD. Since one possible metabolic transformation of CBD could be a cyclization to Δ^1-THC, the extract was analysed for Δ^1-THC by thin-layer chromatography (Korte & Sieper, 1960) and gas chromatography. Any formed Δ^1-THC could not be detected.

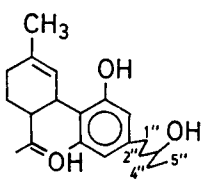
Further extractions of the incubate with diethyl ether recovered several metabolites (35% conversion of CBD) which were partially separated by column chromatography on Florisil (*cf.* Nilsson & others, 1970). One fraction of similar polarity to 7-hydroxy-Δ^1-THC was further purified by t.l.c. on Silica gel G plates with ether–light petroleum (5:1) as solvent. The isolated material (2 mg) was found by g.l.c. to contain, apart from minor constituents, two metabolites (II and III) in the ratio 8:2.



I



II



III

The mass spectrum of the main metabolite (II) showed prominent peaks at *m/e* (rel. intensity): 330 (7, M^+), 315 (3), 312 (35), 299 (37), 244 (100), and 231 (51). The peak at *m/e* 299 is formed by the loss of $-CH_2OH$. This is also a typical feature in the spectrum of 7-hydroxy-Δ^1-THC (Nilsson & others, 1970) and indicates a hydroxylation of CBD at C-7 or possibly C-10. The nmr spectrum of the metabolite mixture, containing 80% of II, showed a singlet at δ 1·68 due to the presence of the C-10 methyl group (in CBD at δ 1·67 ppm) with only a minor peak at δ 1·82 indicative of the C-7 methyl group in III. A signal at δ 4·02 (singlet), roughly equivalent to two protons, is assigned to the $-CH_2O-$ group of II (at δ 4·02 in 7-hydroxy- Δ^1-THC). On the basis of this evidence the main metabolite of CBD (II) is assigned the structure 7-hydroxy-CBD. The C-7 hydroxylation of CBD is analogous to the primary C-7 hydroxylation of Δ^1-THC and CBN (*cf.* Agurell, Dahmén & others, 1972).

* Designated as Δ^9-THC using the benzopyran numbering system.

† 11-Hydroxy- Δ^9-THC.

The mass spectrum of metabolite III showed prominent peaks at *m/e* (rel. intensity): 330 (9, M^+), 315 (4), 297 (3), 262 (26), 247 (100), and 245 (6). The presence of the fragment *m/e* 247 (base peak) instead of *m/e* 231 as in the spectrum of CBD (Budzikiewicz, Alpin & others, 1965) implied that a hydroxyl group was located in the pentyl side-chain. 1″-Hydroxy-CBD, 2″-hydroxy-CBD and 3″-hydroxy-CBD were synthesized and compared with compound III by g.l.c. t.l.c. and mass spectrometry. The syntheses of 1″-hydroxy-CBD and 2″-hydroxy-CBD are discussed in a recent review (Agurell & others, 1972). 3″-Hydroxy-CBD was synthesized by the reaction of 1-(3,5-dihydroxyphenyl)pentan-3-one ethylene dithioketal (Fahrenholtz, 1972) and (+)*trans-p*-mentha-2,8-dien-l-ol in the presence of *NN*-dimethyl-formamide dineopentyl acetal. The resulting 3″-ethylene dithioketal derivative of CBD was treated with mercuric chloride and cadmium carbonate, followed by sodium borohydride to yield 3″-hydroxy-CBD.

G.l.c. (3% SE-30/Gas-Chrom Q, 200°) gave a retention time for 1″-hydroxy-CBD of 6·45 min, for 2″-hydroxy-CBD 6·20 min, for 3″-hydroxy-CBD 6·70 min and for III 6·70 min. By t.l.c. (alumina plates eluted with $CHCl_3$ and silica gel plates eluted with ether: light petroleum 1 : 1) III had the same R_F-value as 3″-hydroxy-CBD. Also, the mass spectrum of III was in agreement with that of the synthetic 3″-hydroxy-CBD. Thus, 3″-hydroxy-CBD is indistinguishable from metabolite III by t.l.c., g.l.c. and mass spectrometry.

In this connection it may be noted that $\Delta^{1(6)}$-THC hydroxylated in the side chain at the 1″- and 3″-position, respectively, have been isolated from *in vitro* experiments with $\Delta^{1(6)}$-THC using dog liver (Maynard, Gurney & others, 1971).

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