In vivo metabolites of $\Delta^{1(6)}$-tetrahydrocannabinol produced by the mouse via the epoxide-diol pathway

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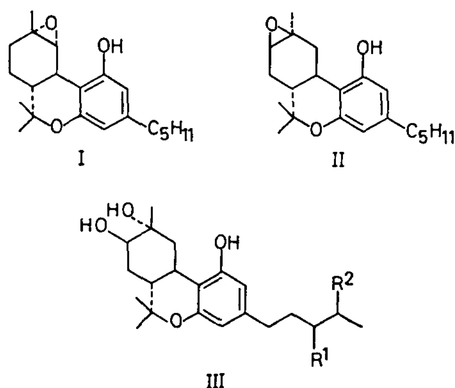
An epoxide metabolite of Δ^1-tetrahydrocannabinol (Δ^1-THC) was first reported by Gurny, Maynard & others (1972) from a monkey liver microsomal preparation and was shown to be 1α,2α-epoxyhexahydrocannabinol (I) by Mechoulam, Varconi & others (1972). More recently, this compound has also been identified as a metabolite of Δ^1-THC in the isolated, perfused dog lung (Widman, Nordqvist & others, 1975) and from a rabbit microsomal preparation (Ben-Zvi & Burstein, 1975). We now report the characterization of a similar epoxide (II), together with the derived diol, 1α,6β-dihydroxyhexahydrocannabinol (IIIa) and its 3″-, (IIIb) and 4″-hydroxy (IIIc) derivatives as *in vivo* liver metabolites of $\Delta^{1(6)}$-THC in the mouse.



IIIa $R^1 = R^2 = H$
IIIb $R^1 = OH$ $R^2 = H$
IIIc $R^1 = H$ $R^2 = OH$

Male Charles River CD1 mice (23 g) were treated intraperitoneally with $\Delta^{1(6)}$-THC (100 mg kg^{-1}) suspended in Tween 80 and isotonic saline 1 h before death by stunning and decapitation. The metabolites were extracted from the homogenized livers with ethyl acetate, separated from endogenous lipids by chromatography on Sephadex LH-20, and examined by combined gas-liquid chomatography-mass spectrometry (Varian 2400 GLC, V.G. Micromass 12B mass spectrometer and a V.G. 2040 data sytem) as described previously (Harvey & Paton, 1976a,b). The g.l.c. column (3% SE-30) was temperature programmed from 190° to 290° at 2° min^{-1} and mass spectra were recorded at 25 eV.

Fraction 2 from the Sephadex LH-20 column (18–26 ml $CHCl_3$) contained, in addition to unmetabolized $\Delta^{1(6)}$-THC, trace amounts of an epoxide metabolite. Its trimethylsilyl (TMS) derivative had a retention time (6·3 min) and a mass spectrum [$M^{+\cdot}$, *m/e* 402 (56%), major fragment ions at *m/e* 387 (38% $(M-CH_3)^+$), 384 (22% $(M-H_2O)^{+\cdot}$), 369 (14%), 359 (16%) and 343 (100%)] identical with those of 1β,6β-epoxy-hexahydrocannabinol (II) synthesized by the reaction of $\Delta^{1(6)}$-THC with *m*-chloroperbenzoic acid as described by Ben-Zvi, Mechoulam & others (1971) and Mechoulam, Ben-Zvi & others (1973). No reaction of the epoxide with the TMS reagents was noted although this has been observed to occur rapidly with oxides such as cyclohexane oxide and oxides produced metabolically from allylic barbiturates (Harvey, Glazener & others, 1972a; Harvey, Johnson & Horning, 1972b).

Epoxides have been shown to be intermediates in the production of several metabolites such as diols, phenols, glutathione conjugates and mercapturic acids from many aromatic and olefinic substrates (Maynert, Foreman & Watabe, 1970; Daly, Jerina & Witkop, 1972) and thus such compounds, particularly diols, might also be expected as metabolites of $\Delta^{1(6)}$-THC. Fraction 5 from the Sephadex LH-20 column (10% $MeOH-CHCl_3$) contained a dihydroxy metabolite whose per-TMS derivative had a molecular weight of 564 [27 a.m.u. shift in the [$^2H_9$]TMS spectrum (McCloskey, Stillwell & Lawson, 1968)]. A high resolution mass measurement (V.G. Micromass 70/70F, probe inlet) of the underivatized sample [$M^{+\cdot}$, *m/e* 348·2295 ($C_{21}H_{32}O_4$: 348·2300)] confirmed the presence of the two additional hydroxyl groups and the absence of the double bond.

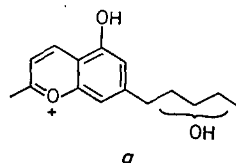
This metabolite was shown to be the *trans*-diol, 1α,6β-dihydroxyhexahydrocannabinol (IIIa) by synthesis as follows. The two *trans*-diols, 1α,6β- and 1β,6α-dihydroxyhexahydrocannabinol, were synthesized in unequal amounts by perchloric acid catalysed hydrolysis of 1β,6β-epoxyhexahydrocannabinol (Ben-Zvi & others, 1971). Each compound formed two TMS derivatives depending on the reaction conditions; and it was found that it was the two derivatives of the major synthetic isomer (82%) that had g.c.-ms properties identical to those of the derivatized metabolite. Thus, with *N,O*-bistrimethylsilyltrifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) the major diol gave the *bis*-TMS ether [Rt 13·3 min, $M^{+\cdot}$ *m/e* 492 (62%), fragment ions at *m/e* 474 (13% $(M-H_2O)^{+\cdot}$), *m/e*, 459 (33%), *m/e* 343 (100%)] containing an underivatized 1α-hydroxyl group, whereas when 1-trimethylsilylimidazole (TMSI) was added to the reaction mixture, the sterically hindered 1α-hydroxyl group reacted to give the *tris*-TMS ether [Rt 15·25 min, $M^{+\cdot}$, *m/e* 564 (11%), major frag-

* Correspondence.

ment ions at *m/e* 474 (39%), 459 (40%), 384 (9%) and 343 (100%)].

Although Ben-Zvi & others (1971) did not report their full experimental conditions for the opening of the epoxide, the properties of our major product indicated that we had obtained the two isomers in a similar ratio with the 1α,6β-diol predominating. The diaxial nature of our major isomer was shown by its failure to form a stable cyclic boronate ester, in contrast to the minor isomer; its slower rate of reaction with TMSI compared with that of the other isomer; and the abundant losses of trimethylsilanol (90 a.m.u.) in the mass spectra of its *tris*-TMS derivative. The latter property was a reflection of the more favourable positioning of the trimethylsilyl groups and hydrogen atoms than existed in the di-equatorial isomer whose spectrum [$M^{+\cdot}$, *m/e* 564 (64%), 474 (47%), 459 (76%), 448 (24%), 433 (83%), 343 (100%)] contained many more abundant ions arising from competing reactions. When the 3α-, 1″-, and aromatic hydrogens of the major isomer were replaced by deuterium (D_2, 10% Pd on charcoal, CD_3OD, see Block & Djerassi, 1973), the first trimethylsilanol loss proceeded with elimination of one deuterium atom. Only the 3α-deuterium is close enough to the trimethylsilyloxy groups to undergo abstraction, and of the two isomeric diols, only the 1α-group in the diaxial isomer is suitably positioned to abstract the 3α-deuterium. The metabolite must therefore, be the *trans*-diol, 1α,6β-dihydroxyhexahydrocannabinol (IIIa). For comparison, the *cis*-diols (Ben-Zvi, Mechoulam & Burstein, 1970) were synthesized by the reaction of $\Delta^{1(6)}$-THC with alkaline permanganate (Schmidt, Richter & Mühlstädt, 1963) and were shown to have g.c.-ms properties which did not correspond to those of the metabolite.

Two other metabolites related to the diol IIIa were observed later in the chromatogram. Their retention times were similar (22·0 and 22·2 min), each formed two TMS derivatives depending on the reaction conditions thus indicating the presence of a hindered hydroxyl group; and their molecular ions and shifts of the fragment ions in the spectra of the [$^2H_9$]TMS derivatives indicated the presence of a third metabolic hydroxyl function. This was confirmed by a high resolution mass measurement of the mixture of underivatized metabolites [$M^{+\cdot}$, *m/e* 364·2230, ($C_{21}H_{32}O_5$: 364·2249)]. The presence in the spectra of the TMS derivatives of both compounds of the ions *m/e* 391 (*a*) and *m/e* 431, both of which contained the intact phenyl and heterocyclic rings together with the pentyl side-chain showed that the additional hydroxyl group was located in this part of the molecule. The spectra of the two TMS derivatives of the first of these compounds (IIIb) had base peaks at *m/e* 436 and *m/e* 508 respectively corresponding to loss of 144 a.m.u. (TMS–OC_4H_7) from the side-chain and indicating 3″-hydroxy substitution (Binder, Agurell & others, 1974; Wall & Brine, 1976). Similar g.l.c. retention increments were observed between compounds IIIa and b as between other metabolites



a

such as $\Delta^{1(6)}$-THC-7-oic acid and their 3″-hydroxy derivatives. The g.l.c. retention increments between the 3″-hydroxy metabolite IIIb and compound IIIc and the presence of *m/e* 117 ($TMS\overset{+}{O}$ = CH–CH_3) in low abundance (12%) in the spectra of the derivatives of the latter compound enabled the third hydroxyl group to be located in the 4″-position (IIIc, Harvey, Martin & Paton, 1977). Although the stereochemistry of the 1- and 6-hydroxyl groups was not determined, the identity of this with that of the diol (IIIa) was indicated by the failure of the compounds to form stable cyclic boronates. Full g.c.-ms data and details of the preparative methods will be published later.

Although there is little doubt that the diol (IIIa) and the triols (IIIb, c) were derived from the corresponding epoxides, the relative amounts produced by the action of hepatic epoxide hydrase or by non-enzymatic hydrolysis are uncertain. Oesch, Kaubisch & others (1971) have reported that some methyl substituted alicyclic epoxides (a grouping similar to that present in these cannabinoid epoxides) are poor substrates for the enzyme but no general conclusions were reached on the relationship between structure and reaction rate. The general stability of the synthetic epoxides under the conditions used for the extraction and isolation of the metabolites, however, would indicate that most of the observed diol was produced metabolically. In any case, the total amounts of the diol and epoxide metabolites are a reflection of the amounts of unconjugated metabolites produced via the epoxide pathway. In the case of $\Delta^{1(6)}$-THC in mouse liver, the diol (IIIa) and triols (IIIb,c) represented about 3 and 1% respectively of the metabolites extractable with ethyl acetate (based on g.l.c. peak areas) with the diol (IIIa) present at a concentration of about 40% that of the major metabolite ($\Delta^{1(6)}$-THC-7-oic acid). As epoxides are known to react with a number of nucleophiles including several macromolecular tissue constituents such as DNA, there remains the possibility that some of the dose not extracted with ethyl acetate represents covalently bound THC resulting from metabolism via an epoxide. Although the recovery of $\Delta^{1(6)}$-THC metabolites has not been examined, studies with radiolabelled Δ^1-THC suggest that this may be significant. In the mouse, Δ^1-THC also appears to undergo metabolism via this pathway (Harvey & Paton, 1976a); trace amounts of the epoxide have been detected in the liver but the diol has not been found.

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Anticonvulsant interaction of cannabidiol and ethosuximide in rats

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Cannabidiol (CBD), a major component of marihuana, has been shown to have dose-response anticonvulsant activity in laboratory animals (Karler, Cely & Turkanis, 1973) and to be devoid of typical marihuana-like psychoactivity in man (Hollister, 1973; Karniol, Shirakawa & others, 1974). Furthermore, CBD is a potent inhibitor of the hepatic microsomal drug-metabolizing enzymes in rodents (Fernandes, Warning & others, 1973). These previous findings and a recent survey showing high incidence of marihuana usage among young epileptics (Feeney, 1976) led us to explore the combined effects of CBD and various standard antiepileptic drugs against electrically- and sound-induced seizures in rats (Consroe & Wolkin, 1977). In the latter study, one anti-epileptic drug, ethosuximide, failed to elicit a graded dose-response effect in either of the two seizure tests. We now report additional data on the interactive effects of CBD and ethosuximide against leptazol-induced minimal (clonic) seizures in rodents. Comparative data are also presented on the interactive effects of ethosuximide, which is extensively metabolized *in vivo* (Chang, Burkett & Glazko, 1972), and an inhibitor of hepatic microsomal enzymes, SKF 525A.

* Correspondence.

CBD was incorporated into a 10% polysorbate (Tween 81) 80–0·9% saline vehicle and ethosuximide, SKF 525A (β-diethylaminoethyldiphenylpropylacetate hydrochloride) and leptazol were dissolved in 0·9% saline. Male Sprague-Dawley rats (200–300 g) were treated in groups of ten with one of the following: Tween 81-saline (2 ml, oral), saline (1 ml, i.p.), ethosuximide (250 mg kg^{-1}, oral), CBD (34 mg kg^{-1}, oral), CBD (17 mg kg^{-1}, i.v.), and SKF 525A (10 mg kg^{-1}, i.p.). Each treatment was followed by leptazol (70 mg kg^{-1}, s.c.) at 1, 2, 4, and 8 h intervals. Of all compounds given, only ethosuximide blocked leptazol-induced minimal seizures and showed a maximum effect at 1 h. A median effective dose (ED50) of ethosuximide against leptazol seizures was then determined by the method of Litchfield & Wilcoxon (1949). Subsequently the ED50 of ethosuximide was determined in different animals 1 h after the drug had been given concurrently with CBD (34 mg kg^{-1}, oral) or SKF 525A (10 mg kg^{-1}, i.p.). Anticonvulsant potency comparisons among ED50 of ethosuximide alone and with the two other agents showed significant differences ($P < 0{\cdot}05$) of the following order: SKF 525A + ethosuximide > ethosuximide > CBD + ethosuximide. The ED50 and 95% confidence limits (in mg kg^{-1}) were: SKF 525A + ethosuximide,