The free radical oxidation of the tetrahydrocannabinols

I.J. Miller, N.K. McCallum, C.M. Kirk and B.M. Peake

Chemistry Division, Department of Scientific and Industrial Research, Private Bag, Petone (New Zealand), and Chemistry Department, University of Otago, Dunedin (New Zealand), 11 May 1981

Summary. The free radical oxidation of Δ^1- and Δ^6-tetrahydrocannabinol has been examined by spin trapping techniques and intermediates that would lead to cannabinol have been trapped. The 1st step in the oxidation of Δ^1-THC involves the removal of 3-H, while for Δ^6-THC, either 2-H or 5-H. Intermediates were isolated which could be pyrolysed to the diene and cannabinol.

Both naturally-occurring tetrahydrocannabinols (Δ^1-THC, Δ^6-THC; fig. 1) have been shown to be transformed to cannabinol (CBN, fig. 1) in vivo[1-3], but some in vitro systems (including aerial oxidation and oxidation with chloranil) convert only the Δ^1-isomer to CBN[4,5]. This difference has been assumed to arise from only the Δ^1-isomer possessing allylic and benzylic activation, and the reaction proceeding via an ionic mechanism. The aerial oxidation has been shown to proceed through 2 diene intermediates[5].

In vivo oxidation of both isomers takes place with equal facility[6]. Burstein[7] has proposed that the 1st step would be formation of the known 7-hydroxy metabolite[8] which could then aromatize to CBN. On the other hand, McCallum[3] observed an isotope effect from the in vivo reaction of Δ^1-THC tritiated at C-3, which would indicate removal of the C-3 proton is the rate-determining step.

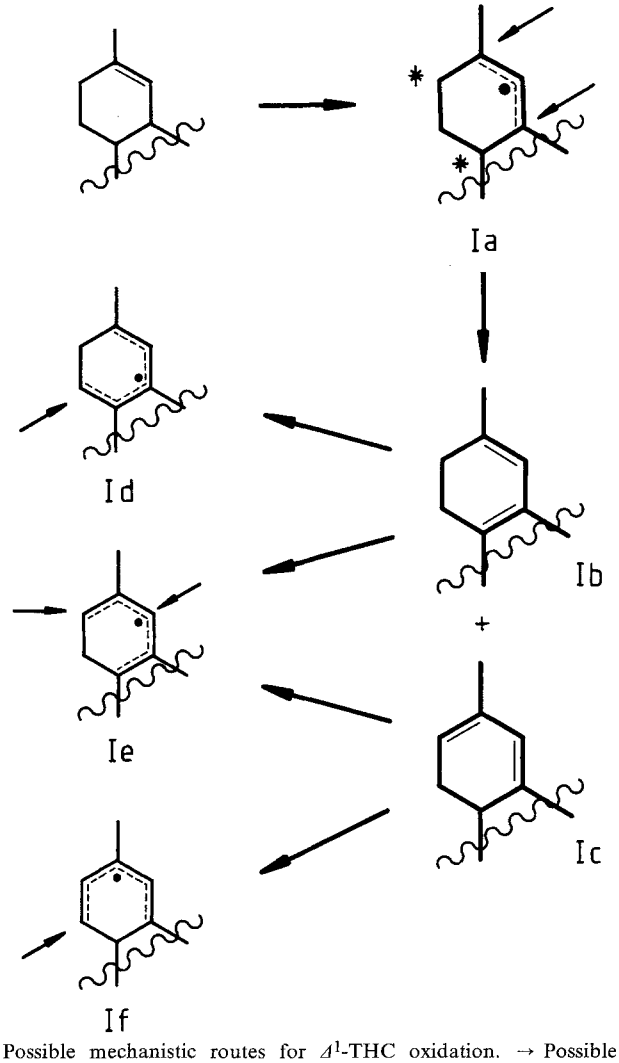
Δ^1-THC appears to be oxidized in vivo by cytochrome P450[9-11], through a postulated ferryl ion. Cytochrome P450 was classically thought to hydroxylate by an insertion mechanism, since in most reactions there is a low isotope effect. However, significant isotope effects have been observed from P450 oxidations at a benzylic site, and Loew suggests that hydrogen abstraction may be the initial step for P450 hydroxylations[12].

Since isotope effects have been observed for the in vivo oxidation of Δ^1-THC, a free radical mechanism cannot be dismissed. Before such a mechanism can be proposed, however, it is necessary to show that the reaction of Δ^1-THC with free radicals proceeds through a pathway involving the dehydrogenation at C_3. Thus we have oxidized both isomers of THC, and we have examined the free radical derivatives.

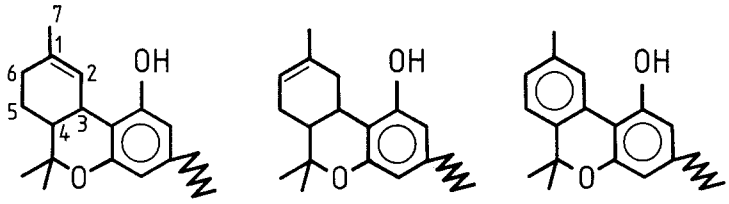
Materials and methods. Di-t-butyl peroxide (Fluka) and benzene (AnalaR) were used without further purification, while acetophenone was redistilled. 2,4,6-tri-t-butyl-nitrosobenzene was prepared and purified as described elsewhere[13]. Δ^1- and Δ^6-THC were purified to > 99.5% purity[8]. ESR-spectra were run on a Varian E4 spectrometer while degassed solutions were irradiated with a 250 W medium pressure mercury lamp.

Results and discussion. When acetophenone was added as a sensitizer to Δ^1-THC in di-t-butylperoxide a weak ESR-signal (half-life 2 sec at −35 °C) was observed. The signal was asymmetric and time dependent which suggests the presence of more than 1 species. The signal at −50 °C was approximately 9 gauss wide and had 8 lines which could not be well resolved. This spectrum was inadequate for assignment purposes. Attempts were then made to trap these radicals with the spin trap 2,4,6-tri-t-butylnitrosobenzene, which was chosen because the resultant spectra cannot only reveal considerable structural information, but also would indicate steric crowding at the trapping site[14] if anilino radicals are formed. Degassed solutions of di-t-butyl peroxide, THC and the spin trap in benzene were irradiated for short periods of time at room temperature and the signal shape changed with time suggesting that a succession of species were being formed and removed.

For Δ^1-THC the 1st signal was weak and similar to a nitroxide spectrum we have observed when phenol was irradiated under these conditions. Following this, a triplet of triplets appeared which had a lower g-value than the above nitroxide. This was assigned to an anilino radical where the trap was linked to a tertiary carbon atom. The hyperfine splitting constants ($a_N = 10.0$ G, $a_{m\text{-}H} = 1.9$ G) correspond closely to similar radicals observed with the trapped t-butyl radical[14]. After further irradiation (time $\approx \times 2$ that required for the 1st anilino radical) this was replaced by a 2nd anilino radical, where the trap was linked to a secondary carbon atom ($a_N = 10.25$ G, $a_{\beta\text{-}H} = 5$ G, $a_{m\text{-}H} = 1.5$ G). The $a_{\beta\text{-}H}$ splitting of 5 G is unusually large for



Possible mechanistic routes for Δ^1-THC oxidation. → Possible trapping site consistent with observed radicals. * Possible sites for the removal of a hydrogen atom.



Cannabinol structures. Δ^1-THC Δ^6-THC CBN

an anilino radical, but we believe that it was an anilino radical because its g-value was similar to the 1st anilino radical, but different from a nitroxide, and the signal was characteristic of an anilino radical[14]. We assume that steric pressure forced the trap into an angle with the β-H conducive to the larger coupling constant.

For Δ^1-THC the proton at the C-3 carbon is the most activated for radical proton abstraction, and the derived radical could only be trapped at a tertiary carbon atom (C-3 or C-1). As the removal of any other proton would also allow trapping at a secondary centre, the absence of any signals corresponding to such a species is good evidence that it is the C-3 proton that is removed. Following this a diene could be formed by the abstraction of either the C-4 or C-6 proton (see scheme I), a dienylic radical would be formed by the abstraction of a further proton from either of these dienes, and superficially this radical might be expected to be trapped at C-5 or C-6, depending on the species. That the dienylic radical is an anilino radical indicates that the species is trapped at a position of considerable steric strain, and we suggest that this could be at C-2. This position may be favoured for trapping, since it involves the least movement of a bulky trap if initial trapping is at C-3.

Further solutions were irradiated in the absence of spin trap. Analysis of the resultant product by gas chromatography (polar and non-polar columns) confirmed the presence of both THC and CBN, showing that dehydrogenation is the predominant reaction, but CBN was not observed on TLC. Analysis of the crude product by mass spectrometry indicated the presence of a 3rd component. GC purification was not possible, as the compound was not observed in the GC trace, presumably because it pyrolysed, but molecular fragments of 383, 382, 366, 351 and 335 were observed. The combination of the proposed dienylic radical and a t-butoxy radical would give a compound of MW 384. The observed fragments could correspond to M-1, M-2 (unexpected), M-18, M-18-15, and M-1-18(2×15). Fragments below 314 (the MW of THC), were not considered relevant, as the sample was not pure, although it was noticeable that there was only a very weak fragment at 295, which is the major fragment for CBN[15]. The pyrolytic elimination of t-butanol from this proposed compound would give CBN in the GC analysis. GC-MS gave a mixture of products of MW 314, 312 and 310, together with a strong fragment at MW 295. This is consistent with the presence of THC, the diene and CBN, the latter 2 being formed by pyrolysis from the postulated addition products to the enylic and dienylic radicals.

Similar irradiation of the Δ^6-THC led to a sequence of spin trapped species. Initially a nitroxide was formed, where the ESR-spectrum suggested that the nitroxide was linked to a secondary carbon atom ($a_N = 13.5$ G, $a_{\beta\text{-H}} = 22$ G). After further irradiation the signal suggested the formation of low concentrations of an anilino radical, trapped at a tertiary carbon atom ($a_N = 10.04$ G, $a_{m\text{-H}} = 1.85$ G).

Since the initial trapped species for Δ^6-THC were also nitroxides, trapping occurred at sites less sterically hindered than for Δ^1-THC. This indicates that the initial trapping occurs at C-5 or C-6. That a subsequent species was an anilino radical with no β-H splitting shows that a subsequently-oxidized species was trapped, at least in part, at a tertiary centre. The ESR-signals from the nitroxide species were sufficiently broad so that more than 1 nitroxide species could have been present. Further, it could be assumed to be too difficult to differentiate between the trapped nitroxides of an allylic and dienylic radical, and hence the fact that the levels of anilino radical were low is probably indicative of only a small fraction of the dienylic radicals were being trapped at a tertiary position.

Conclusions. This study provides good evidence that the initial step in the free radical oxidation of Δ^1-THC is the removal of the C-3 hydrogen atom. The observed isotope effect[3] indicates that this is the 1st step for enzymatic in vivo oxidations also. Further, free radical oxidation consistent with the formation of dienes, then dienylic radicals, demonstrates that the path to CBN follows that outlined by Razdan[5]. Oxidation of Δ^6-THC follows a similar dehydrogenation path, although the initial oxidation is at a different site.

Thus, the broad features of a free radical oxidation are clear, and both species are oxidized by a dehydrogenation which would lead to CBN. These results show that oxidation of THC to CBN could occur by a radical mechanism.

1 M. Widman, M. Nordquist, S. Agurell, J.E. Lindgren and F. Sandberg, Biochem. Pharmac. *23*, 1163 (1974).
2 D.E. Green, in: Cannabinoid Assays in Humans, p.20. Ed. R.E. Willette. NIDA Res. Mon. Ser. 7, 1976; S. Burstein and C. Varanelli, Res. Commun. chem. Path. Pharmac. *11*, 343 (1975).
3 N.K. McCallum, A. Gugelmann, C.A.M. Brenninkmeijer and R. Mechoulam, Experientia *33*, 1012 (1977).
4 Marijuana. Chemistry, Pharmacology, Metabolism and Clinical Effects. Ed. R. Mechoulam. Academic Press, New York 1973.
5 R.K. Razdan, A.J. Puttick, B.A. Zitko and G.R. Handrick, Experientia *28*, 121 (1972).
6 N.K. McCallum, B. Yagen, S. Levy and R. Mechoulam, Experientia *31*, 520 (1975).
7 R. Mechoulam, N.K. McCallum and S. Burstein, Chem. Rev. *76*, 102 (1976).
8 L. Lemberger, Drug Metab. Dispos. *1*, 461 (1973).
9 D. Kupfer, I. Jansson, S. Orrenius, Chem.-Biol. Interact. *5*, 201 (1972).
10 J.V. Dingell, K.W. Miller, E.C. Heath and H.A. Klausner, Biochem. Pharmac. *22*, 949 (1973).
11 G.M. Cohen, D.W. Peterson and G.J. Mannering, Life Sci. *10*, 1207 (1971).
12 L.M. Hjelmeland and G.H. Loew, J. Am. chem. Soc. *99*, 3514 (1977).
13 R. Okazaki, T. Hosagai, E. Iwadare, M. Hashimoto and N. Inamoto, Bull. chem. Soc. Japan *42*, 3611 (1969).
14 S. Terabe and R. Konaka, J. chem. Soc. Perkin *2*, 369 (1973).
15 S.R. Heller and G.W.A. Milne, EPA/NIH Mass Spectral Data Base, NSRDS-NBS 63 (1978).