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176. Microbial Transformation of (–)-Δ^1-3,4-*trans*-Tetrahydrocannabinol by *Cunninghamella blakesleeana* LENDER

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Summary. Incubation of (–)-Δ^1-3,4-*trans*-tetrahydrocannabinol (= Δ^1-THC; **3**) with stationary cultures of *Cunninghamella blakesleeana* LENDER (*Zygomycetales*) (ATCC 8688a) yielded a number of metabolic conversion products. Isolation and structure elucidation of 6α-hydroxy-Δ^1-THC (**4**), the potential psychoactive 3″-hydroxy-Δ^1-THC (**2**) and 4″-hydroxy-Δ^1-THC (**1**), and the hitherto unknown metabolites 4″-hydroxy-6-oxo-Δ^1-THC (**5**), 4″,6α-dihydroxy-Δ^1-THC (**7**) and 4″,7-dihydroxy-Δ^1-THC (**6**) is described.

1. Introduction. – Since the first major metabolite of (–)-Δ^1-3,4-*trans*-tetrahydrocannabinol[1]) (= Δ^1-THC; **3**), 7-hydroxy-Δ^1-THC, was isolated in 1970 from *in vitro* incubations using a rat liver microsomal preparation [2] [3], a number of hydroxylated or oxygenated metabolic conversion products of Δ^1-THC (**3**) have been described. These include 6α-hydroxy-Δ^1-THC (**4**), 6β-hydroxy-Δ^1-THC, 1,2α-epoxy-Δ^1-THC, 6-oxo-Δ^1-THC and 7-oxo-Δ^1-THC. Recently *Widman et al.* [4] reported the isolation of two sidechain hydroxylated metabolites of Δ^1-THC (**3**), 3″-hydroxy-Δ^1-THC (**2**) and 4″-hydroxy-Δ^1-THC[1a]) (**1**), formed by conversion of **3** in the perfused dog lung.

It has been demonstrated by *Perez-Reyes et al.* [5], that two of the monohydroxylated metabolites, 7-hydroxy-Δ^1-THC and 6β-hydroxy-Δ^1-THC are of considerable psychotropic activity in man, the former being equipotent, the latter about 1/3 as potent as Δ^1-THC (**3**). Synthetically prepared sidechain hydroxylated derivatives of Δ^6-THC [6] have been administered to rhesus monkeys [7]. This seems to be a suitable laboratory animal to correlate THC induced behavioral changes with psychotropic activity. The results indicate that, compared to Δ^6-THC, 3″-hydroxy-Δ^6-THC is several times as potent while 5″-hydroxy-Δ^6-THC is about equipotent and

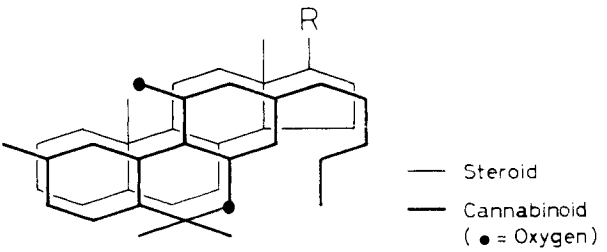
[1]) The monoterpenoid numbering for cannabinoids [1] (*cf. Scheme 2*) is used in this paper: Δ^1-THC corresponds to Δ^9-THC of the IUPAC nomenclature.

[1a]) *Added in proof:* Independent of our work, the formation of 4″-hydroxy-Δ^1-THC (**1**) by biotransformation of Δ^1-THC (**3**) has been reported by *Robertson et al.* [21].

2″-hydroxy-Δ^6-THC somewhat less potent. 1″-hydroxy-Δ^6-THC lacked psychotropic activity in the rhesus monkey. 4″-hydroxy-Δ^6-THC, which was not available for testing would be expected to range between the 3″- and 5″-hydroxy compounds.

Regarding the fact, that in man Δ^1-THC (**3**) is one and a half times as potent as Δ^6-THC in producing a subjective 'high' on oral administration [8], one would expect sidechain hydroxylated metabolites of Δ^1-THC (**3**) to be of considerable psychotropic activity. The possible significance of such metabolites in man has recently been discussed by *Agurell et al.* [6]. While synthesis of 6α-hydroxy-Δ^1-THC (**4**), 6β-hydroxy-Δ^1-THC and 7-hydroxy-Δ^1-THC in the gram scale has been accomplished [9], no synthesis of sidechain hydroxylated derivatives of Δ^1-THC (**3**) has been reported so far. Minute amounts of metabolites (*e.g.* **1** and **2**) obtained from *in vitro* experiments [4] do not allow any pharmacological evaluation of their activity.

In order to prepare larger quantities of sidechain hydroxylated derivatives of Δ^1-THC (**3**) we have chosen a microbiological approach. Microorganisms have been used for their ability to hydroxylate complex organic molecules, especially steroids. A formal superposition of the carbon-oxygen skeleton of Δ^1-THC (**3**) and the carbon skeleton of steroids suggests the possibility of microbial transformations of cannabinoids.



Several microorganisms were tested for their capacity to hydroxylate Δ^1-THC (**3**). Among these the *zygomycete Cunninghamella blakesleeana* LENDER, which is well known for its ability to hydroxylate steroids in the 6α, 9α, 11β and 14α position [10–12] gave the best results and was chosen for further investigations.

We wish to report on the isolation of two sidechain hydroxylated cannabinoids, 3″-hydroxy-Δ^1-THC (**2**) and 4″-hydroxy-Δ^1-THC (**1**), and several other metabolic conversion products of Δ^1-THC from incubations with *C. blakesleeana*. Because microbial fermentations are not subjected to the limitations of *in vitro* and *in vivo* experiments with animals and tissue homogenates, it will be possible to produce larger amounts of these metabolites and to evaluate their pharmacological activity.

2. Incubation of Δ^1-THC (3). – The *zygomycete Cunninghamella blakesleeana* LENDER (ATCC 8688a) was cultivated on mycophilic agar slants or plates. Submerse cultures, inoculated with a spore suspension from agar plates, were prepared in corn-steep liquor-[2]), malt- and *Czapek-Dox*-medium. The growth of *C. blakesleeana* was measured by determination of the dry weight of the mycelium. The pH of the cultures was found to range between 5 and 7. Under these conditions no acid catalysed isomerization or decomposition of **3** would be expected.

[2]) The author is indebted to the *Maizena AG*, Krefeld, for a gift of cornsteep liquor.

Δ^1-THC (**3**) was synthesized according to *Petrzilka et al* [13][3]) and tritiated at C(3′) and C(5′) [14] to give a preparation (specific activity 0,24 mCi/mmol) which was 97% pure. Gas liquid chromatography (GLC.) revealed a 3% impurity consisting of Δ^6-THC.

Δ^1-THC (**3**) (stored in ethanol at $-20°$ in the dark) was added to cultures of *C. blakesleeana* (5 mg/50 ml culture) at different times after inoculation and was incubated for periods ranging from 24 to 120 h in the dark. Culture filtrate and mycelium were extracted with ethyl acetate and methanol, respectively. The recovery of Δ^1-THC (**3**) and metabolites was determined by liquid scintillation counting and averaged 95%. Thin layer chromatography (TLC.) was used to compare the extracts with reference compounds[4]) (6α-hydroxy-Δ^1-THC (**4**), 6β-hydroxy-Δ^1-THC, 7-hydroxy-Δ^1-THC and 6α,7-dihydroxy-Δ^1-THC) and the spots were visualized with *Fast Blue Salt B* reagent allowing an easy discrimination between cannabinoids and nonphenolic material extracted from the cultures.

As blanks an extract of a *C. blakesleeana* culture, a Δ^1-THC sample that had been incubated with a stationary sterilized culture of the microorganism, and – in order to eliminate decomposition products [15] – a sample of Δ^1-THC (**3**) that had been treated in H_2O/ethanol 95:5 at pH 4 during the time corresponding to the longest actual incubation time, were used.

As judged from TLC. the pattern of metabolic conversion products formed by *C. blakesleeana* was the same for the three media employed, but the yield of metabolites was best for the cornsteep liquor medium which was used for further incubations. Incubation for 24 to 48 h with stationary cultures of *C. blakesleeana* favored the formation of monohydroxylated metabolites, while incubation for 72 to 96 h led to the formation of more polar material.

3. Isolation of the metabolites. – The extracts of culture filtrate and mycelium were combined and chromatographed on *Florisil* (*Scheme 1*). Batch elution gave a rough separation in starting material, mono- and dihydroxylated metabolites.

Fraction 1 was shown by TLC. and GLC. to contain unchanged Δ^1-THC (**3**). Fraction 2 consisted of two major metabolites and was rechromatographed on *Sephadex* LH-20. Metabolites **1** and **4** were well separated, but TLC. revealed the presence of the minor metabolite **2**, which on *Sephadex* LH-20 had the same rentention volume as **1**. Separation of metabolites **1** and **2** and purification of **4** were achieved by preparative TLC. (prep. TLC.). From fraction 3, which contained traces of **1**, **4** and some more polar material, metabolite **5** was isolated by repeated prep. TLC. According to GLC. all metabolites contained impurities (up to 5%), but no further purification was attempted regarding the loss of material on prep. TLC. Rechromatography of fraction 4 on *Florisil*, using a flat solvent gradient resolved metabolites **6** and **7** which were purified again by prep. TLC. The latter metabolites could not be volatilized for GLC. analysis, but were checked in derivatized form (as trimethylsilyl ethers).

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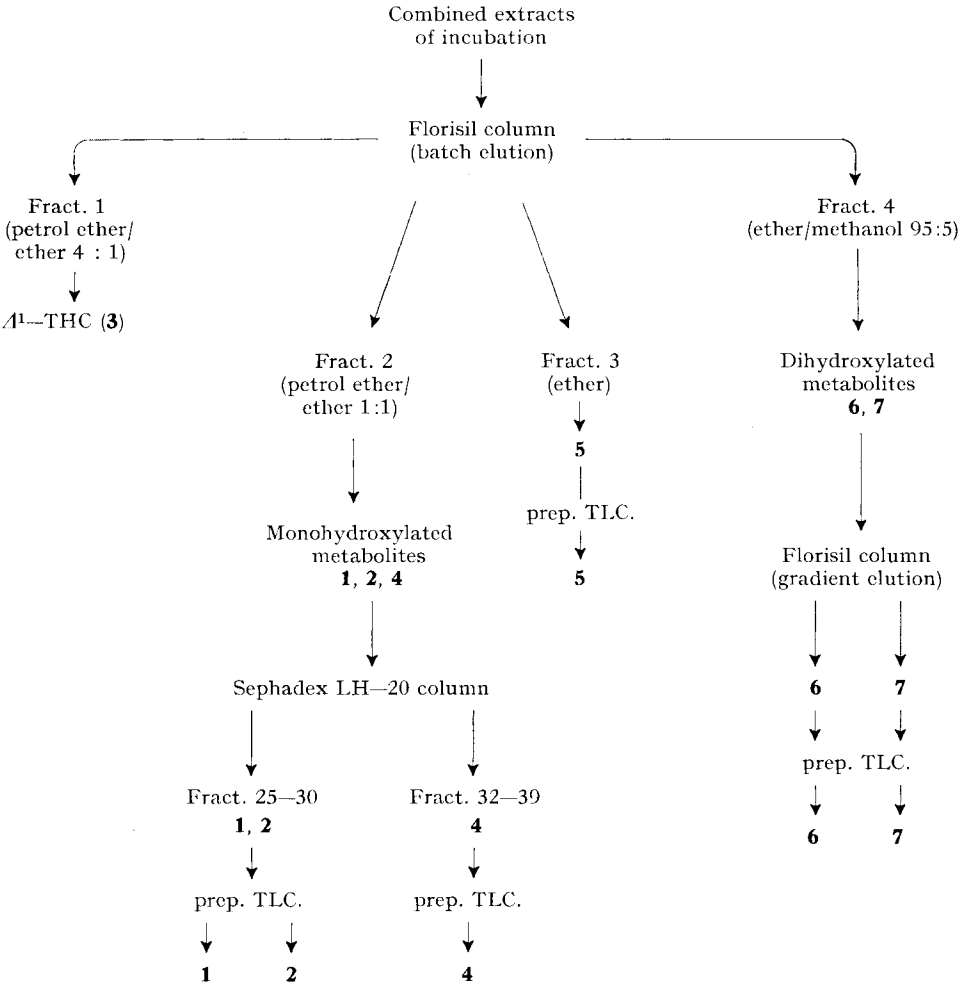
[3]) The author wishes to thank *Firmenich S.A.*, New York, for a generous gift of (+)-*trans*-*p*-mentha-2,8-dien-1-ol.

[4]) The author is greatly indebted to Dres. *S. Agurell*, Uppsala, and *R. Mechoulam*, Jerusalem, for valuable gifts of reference compounds.

Scheme 1. *Separation of metabolites of Δ^1-THC* (**3**) *from culture filtrate and mycelium extracts of* C. blakesleeana



The amounts of metabolites from an incubation of 80 mg of Δ^1-THC (**3**) with a 1 l culture of *C. blakesleeana*, calculated by radioactivity measurements and by estimation of the GLC. peak area, are given in Table 1.

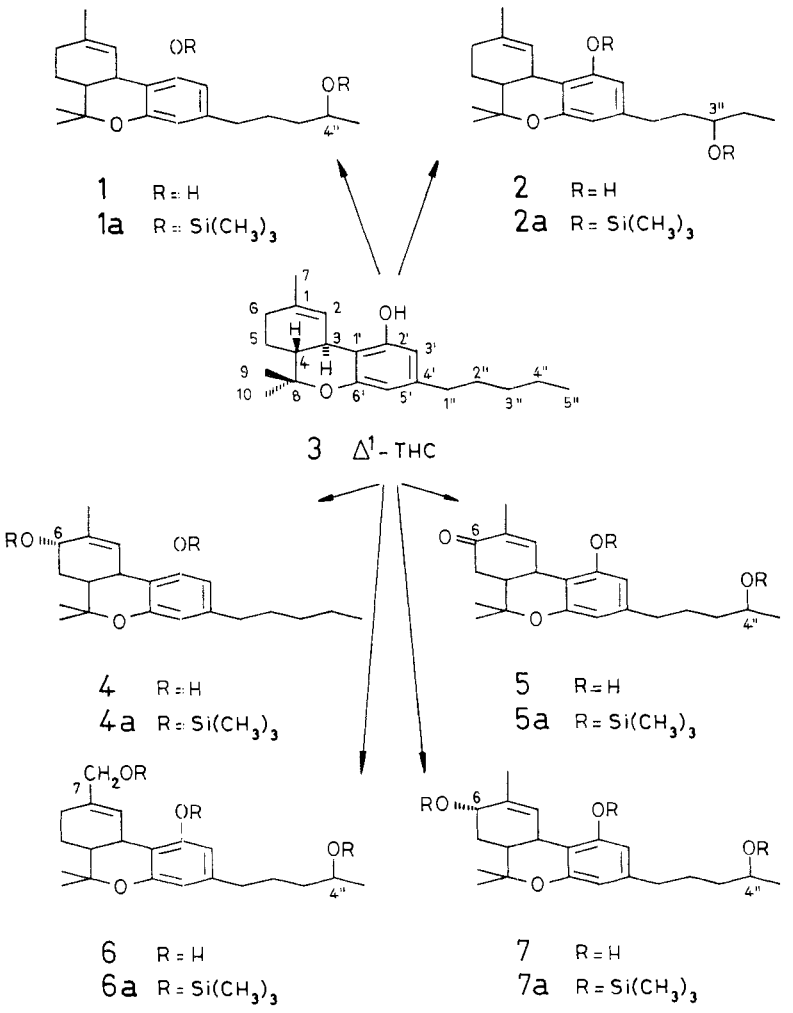
Table 1. *Yield of metabolites of Δ^1-THC* (**3**) *from incubation with* C. blakesleeana

Metabolite	**1**	**2**	**4**	**5**	**6**	**7**
Yield (μg) [a]	1380	64	164	113	700	2360
Metabolic conversion (%)	1.72	0.08	0.21	0.14	0.87	2.95

[a] Obtained from 80 mg of Δ^1-THC (**3**).

4. Structures of the metabolites. – The structures of the metabolites (*Scheme 2*) were elucidated by mass spectroscopy of the compounds and their trimethylsilyl ethers[5]). With metabolites **1**, **6** and **7**, the isolated amounts were sufficient to confirm the assigned structures by ^{1}H-NMR. spectroscopy[6]) The mass spectra (MS.) of cannabinoids [16] [17] and the fragmentations of sidechain hydroxylated cannabinoids and their trimethylsilyl ethers have been discussed in detail [18].

Scheme 2



[5]) The author is indebted to Dr. *D. Müller*, Dept. of mass spectroscopy, Ruhr-University Bochum, for running the mass spectra.

[6]) The author wishes to thank Dr. *W. Dietrich*, Dept. of NMR. spectroscopy, Ruhr-University Bochum, for recording the NMR. spectra.

Metabolite **1** gave a strong molecular ion at *m/e* 330 indicating a monohydroxylated tetrahydrocannabinol. Loss of a methyl group and *Retro-Diels-Alder* cleavage of the isoprenoid part of the molecule led to the ion *m/e* 247. The fragment *m/e* 174 resulting from the loss of 73 mass units (C_4H_9O) is characteristic for a sidechain hydroxyl group (Table 2). The position of the HO-group on C(4″) followed from the MS. of the trimethylsilyl ether **1a**, which gave a prominent peak at *m/e* 117 resulting

Table 2. *Mass Spectra of the Metabolites*

Metabolite	Fragment *m/e*[a]		Structure
1	330	(100)	M^+
4″-hydroxy-	315	(58)	$M^+ - CH_3$
Δ^1-THC	312	(32)	$M^+ - H_2O$
	247	(47)	$M^+ - CH_3$ and *RDA*.[b]
	174	(22)	$M^+ - CH_3$, *RDA* and $-C_4H_9O$
1a	474	(6)	M^+
Trimethylsilyl-	391	(1)	$M^+ - CH_3$ and *RDA*.
ether	117	(12)	$C_2H_4OSi(CH_3)_3^+$
2	330	(36)	M^+
3″-hydroxy-	301	(5)	$M^+ - C_2H_5$
Δ^1-THC	258	(100)	$M^+ - C_4H_8O$
	247	(17)	$M^+ - CH_3$ and *RDA*.
2a	474	(22)	M^+
Trimethylsilyl-	391	(2)	$M^+ - CH_3$ and *RDA*.
ether	343	(5)	$M^+ - C_3H_6OSi(CH_3)_3$
	330	(100)	$M^+ - C_4H_7OSi(CH_3)_3$
	131	(5)	$C_3H_6OSi(CH_3)_3$
4	330	(0)	M^+
6α-hydroxy-	312	(28)	$M^+ - H_2O$
Δ^1-THC	297	(100)	$M^+ - H_2O - CH_3$
	240	(24)	$M^+ - H_2O - CH_3 - C_4H_9$
4a	474	(1)	M^+
Trimethylsilyl-	384	(61)	$M^+ - Si(CH_3)_3OH$
ether	369	(9)	$M^+ - CH_3 - Si(CH_3)_3OH$
	343	(10)	$M^+ - 131$
5	344	(12)	M^+
4″-hydroxy-6-	342	(2)	$M^+ - 2$
oxo-Δ^1-THC	327	(100)	$M^+ - 17$
	254	(17)	$M^+ - 17 - C_4H_9O$
5a	488	(52)	M^+
Trimethylsilyl-	445	(10)	$M^+ - C_3H_7$
ether	117	(12)	$C_2H_4OSi(CH_3)_3^+$
6a	562	(16)	M^+
Trimethylsilyl-	547	(6)	$M^+ - CH_3$
ether of	459	(100)	$M^+ - CH_2OSi(CH_3)_3$
4″,7-dihy-	444	(1)	$M^+ - CH_3 - CH_2OSi(CH_3)_3$
droxy-Δ^1-THC	391	(2)	$M^+ - CH_3$ and *RDA*.
	117	(9)	$C_2H_4OSi(CH_3)_3^+$
	103	(4)	$CH_2OSi(CH_3)_3^+$
7a	562	(4)	M^+
Trimethylsilyl-	547	(6)	$M^+ - CH_3$
ether of 4″,6α-	472	(91)	$M^+ - Si(CH_3)_3OH$
dihydroxy-	457	(8)	$M^+ - CH_3 - Si(CH_3)_3OH$
Δ^1-THC	406	(1)	*RDA*.
	391	(3)	$M^+ - CH_3$ and *RDA*.
	117	(10)	$C_2H_4OSi(CH_3)_3^+$

[a] In parentheses the relative intensity.
[b] *RDA*. = *Retro-Diels-Alder* cleavage.

from α-cleavage between C(3″) and C(4″) with charge retention on the silylated fragment. The structure of 4″-hydroxy-Δ^1-THC for metabolite **1** was confirmed by the 90 MHz ^{1}H-NMR. spectrum[7]), which exhibited the predicted signal of H-C(4″) [18] as a quartet (actually the four inner lines of a hextet) centered at $\delta = 3.77$ ppm resulting from equal couplings to the protons at C(3″) and C(5″). The triplet of the terminal methyl group ($H_3C(5'')$) usually appearing at $\delta = 0.9$ has been replaced by a doublet at $\delta = 1.16$ ppm.

The MS. of metabolite **2** gave a molecular ion at *m/e* 330, a M^+-29 fragment indicating α-cleavage between C(3″) and C(4″) and a peak at *m/e* 258 resulting from the

[7]) For a general discussion of the ^{1}H-NMR. spectra of cannabinoids see [18] and [19].

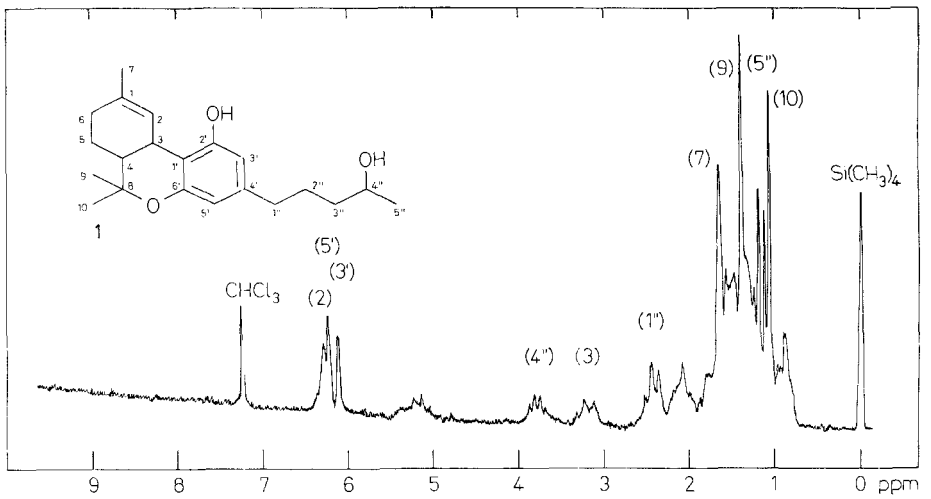


Fig. 1. *90 MHz ^{1}H-NMR. spectrum of 4″-hydroxy-Δ^1-THC* (**1**) *in $CDCl_3$*

loss of the sidechain (cleavage between C(1″) and C(2″) with *McLafferty* rearrangement). Loss of the sidechain with *McLafferty* rearrangement is favored by the presence of groups at C(3″) possessing a negative inductive effect, *e.g.* a HO-group or a $(CH_3)_3SiO$-group. The trimethylsilyl ether **2a** exhibited a base peak at *m/e* 330 (M^+-144) characteristic for such derivatives of 3″-hydroxy-cannabinoids [18]. This fragment corresponds to the ion *m/e* 258 of compound **2**. Additionally, the ions *m/e* 343 and 131 resulting from α-cleavage between C(2″) and C(3″) were observed. Thus the structure of 3″-hydroxy-Δ^1-THC is assigned to metabolite **2**.

Metabolite **4** was on TLC. and GLC. identical with an authentic sample of 6α-hydroxy-Δ^1-THC (**4**). This was confirmed by the MS. of **4** and its trimethylsilyl ether **4a**. 6α-hydroxy-Δ^1-THC (**4**) readily looses water leading in combination with loss of a methyl group to the base peak at *m/e* 297; **4a** gives a typical base peak at *m/e* 384 (M^+-90).

The molecular ion of metabolite **5** at *m/e* 344 indicates a monohydroxylated oxo derivative of Δ^1-THC (**3**). The MS. resembles closely the data published for 6-oxo-Δ^1-THC [20] exhibiting an M^+-2 and M^+-17 (base peak) fragment. The ion at *m/e* 254 arises from the loss of the sidechain containing a HO-group (M^+-17-73). The presence of a HO-group at C(4″) was confirmed by the MS. of the trimethylsilyl ether **5a**, which exhibited a characteristic ion at *m/e* 117. Thus the structure of 4″-hydroxy-6-oxo-Δ^1-THC is assigned to metabolite **5**.

The trimethylsilyl ether **6a** of metabolite **6** gave a molecular ion at *m/e* 562 corresponding to a derivative of a dihydroxylated tetrahydrocannabinol. The intense fragment at *m/e* 459 is characteristic for an silyloxy substituent at C(7) and results from α-cleavage between C(1) and C(7). As was evident from the ion *m/e* 117, the second HO-group of **6** is located on C(4″). The structure of 4″,7-dihydroxy-Δ^1-THC assigned to metabolite **6** was confirmed by the ^{1}H-NMR. spectrum (Fig. 2). The signal of the vinylic proton H–C(2) has been shifted by 0.4 ppm to lower field and the

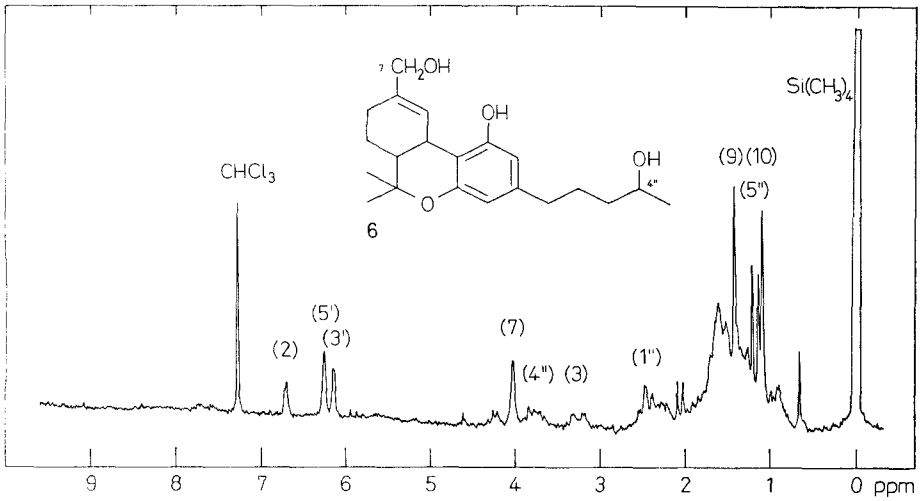


Fig. 2. *90 MHz ^{1}H-NMR. spectrum of 4",7-dihydroxy-Δ^1-THC* (**6**) *in $CDCl_3$*

broad singlet of the protons at C(7) appears now at $\delta = 4.01$ ppm, corresponding to two protons instead of three. The H–C(4") leads to a multiplet at $\delta = 3.8$ and the protons on C(5") give rise to a doublet centered at $\delta = 1.17$ ppm.

The MS. of **7a**, the trimethylsilyl ether of metabolite **7**, exhibited a molecular ion at *m/e* 562 and gave an intense fragment at *m/e* 472 indicating a trimethyl silyloxy substituent at C(6). This fragment (M^+-90) is characteristic for 6α substitution. In the MS. of the trimethylsilyl ethers of 6β-hydroxy-cannabinoids the fragment M^+-90 occurs as well, but it is of low intensity and never gives rise to the base peak. The ions *m/e* 406 and 391 result from the *Retro-Diels-Alder* cleavage of the isoprenoid

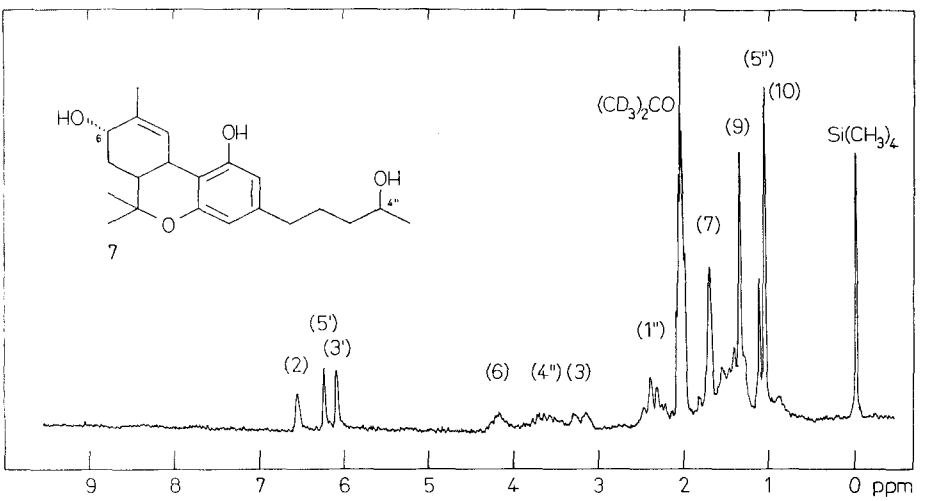


Fig. 3. *90 MHz ^{1}H-NMR. spectrum of 4",6α-dihydroxy-Δ^1-THC* (**7**) *in $(CD_3)_2CO$*

moiety of the molecule and additional loss of a methyl group. Again the ion *m/e* 117 indicates that the second hydroxyl group of **7** must be located on C(4″). The structure of 4″,6α-dihydroxy-Δ^1-THC for metabolic **7** is in agreement with the NMR. data. The H–C(2) appears at $\delta = 6.52$ ppm, the deshielding effect of the allylic HO-group being slightly smaller than in the case of 4″,7-dihydroxy-Δ^1-THC (**6**). A broad multiplet at $\delta = 4.19$ ppm corresponds to H–C(6) in β-position. The H–C(4″) gives rise to a quartet of lines centered at $\delta = 3.69$ and the signal of the protons at C(5″) appears as a doublet at $\delta = 1.10$ ppm, the high field part of which overlaps with the signal of the protons on C(10).

5. Conclusions. – The major route of metabolic conversion of Δ^1-THC (**3**) in *C. blakesleeana* seems to be the introduction of HO-groups in the 6α and 4″ position leading to 6α-hydroxy-Δ^1-THC (**4**) and 4″-hydroxy-Δ^1-THC (**1**). A second hydroxylation can convert both compounds to 4″,6α-dihydroxy-Δ^1-THC (**7**) which could further be oxidized to give 4″-hydroxy-6-oxo-Δ^1-THC (**5**). Hydroxylation at C(3″) is of minor importance and hydroxylation at C(7), a major route of metabolism in animals and humans, occurs only in combination with 4″-hydroxylation, leading to 4″,7-dihydroxy-Δ^1-THC (**6**).

This project was supported by a grant of the *Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen*. The technical assistance of Mrs. *Ch. Risse* and Stud. med. *R. Schwieder* is gratefully acknowledged.

Experimental part

1. *General methods.* Thin layer chromatography (TLC.) and preparative thin layer chromatography (prep. TLC.) were carried out on precoated plates, silicagel 60 F 254, *E. Merck AG.*, Darmstadt. Prep. TLC.-plates were precleaned 3 times with methanol/benzene 1:1. The following solvent systems were used for TLC. and prep. TLC.: *Δ^1-THC* (**3**) 1) petrol ether/ether 95:5 (3×), 2) petrol ether/ether 4:1, 3) $CHCl_3$; *monohydroxylated metabolites* 1) petrol ether/ether 2:3, 2) $CHCl_3$/acetone 4:1, 3) $CHCl_3$/ethyl acetate 4:1; *dihydroxylated metabolites* 1) $CHCl_3$/acetone 3:2, 2) $CHCl_3$/ethyl acetate 3:2, 3) ether, 4) ethyl acetate, 5) ether/methanol 97:3. Spots were visualized with *Fast Blue Salt B* (*Merck*) 0,1% in 0,1 N NaOH or with UV. light. Samples from prep. TLC.-plates were extracted 5 times with CH_2Cl_2/methanol 9:1 or 4:1 at 23°. All solvents (*p.a.* grade, redestilled) were evaporated under dry nitrogen or *in vacuo* at 40°. For column chromatography Florisil (60–100 mesh, *Merck*) and *Sephadex* LH-20 (*Pharmacia Fine Chemicals*, Uppsala) were employed. Gas liquid chromatography (GLC.) was performed on a *Hewlett-Packard*-5711-A gas chromatograph (FID) using columns (1,80 m × 2 mm) of 3% SE-30 or 3% OV-17 on Gaschrom Q at 200–240°. For radioactivity determinations a *Packard Tricarb* model 3380 was used. All mass spectra were recorded at 70 eV ionisation voltage on a *Varian* MAT CH-5 instrument coupled to a *Perkin-Elmer* gas chromatograph model F 20 FE (column 1,20 m × 1,5 mm, 3% SE-30 on Gaschrom Q, 220–240°) and were processed by a *Varian*-SS-100 data system. ^{1}H-NMR. spectra were measured on a *Bruker* WH 90 (FT.) spectrometer. Chemical shifts are given in δ values and refer to $Si(CH_3)_4$ as internal standard, coupling constants *J* in Hz. Trimethylsilyl ethers for mass spectroscopy were prepared by reacting samples of 20 μg of metabolite in 5 μl of acetonitrile with 5 μl of *N,O*-bis-(trimethylsilyl)trifluoracetamide at 60° for 5 to 15 min.

2. *Incubation of Δ^1-THC* (**3**). *C. blakesleeana* was cultivated on mycophilic agar slants and plates. A medium consisting of 10 g of glucose, 30 ml of cornsteep liquor, 0.4 g of $MgSO_4 \cdot 7H_2O$ and 0.4 g of KH_2PO_4 in 1 l of H_2O was distributed in 250 ml portions on 4 1 l Erlenmeyer flasks and inoculated with a spore suspension of *C. blakesleeana*. Four days after incubation at 28° on a reciprocal shaker a solution of 20 mg of Δ^1-THC in 4 ml of ethanol was added to each flask and incubation was continued for 72 h in the dark. The mycelium was filtered off and the culture

filtrate was extracted 3 times with 400 ml of ethyl acetate. The mycelium was homogenized 3 times in a mixer with 100 ml of methanol. The combined extracts were evaporated *in vacuo*, suspended in 100 ml of ethyl acetate and washed with 3 50 ml portions of water to remove inorganic material.

3. *Separation of the metabolites.* The crude extract was separated as summarized in *Scheme 1*. Data of column chromatography: 1) Florisil, 30 g, batch elution; 6 fractions of 250 ml (petrol ether/ether 4:1, petrol ether/ether 1:1, ether, ether/methanol 95:5, ether/methanol 3:2, methanol) were collected at a rate of 10 ml/min. 2) Sephadex LH-20, column 40 cm × 1 cm, eluent $CHCl_3$/petrol ether/ethanol 10:10:1, 60 fractions of 3 ml were collected at a rate of 0.3 ml/min. Fractions 25–30 contained metabolites **1** and **2**, fractions 32–39 metabolite **4**. 3) Florisil, 8 g, gradient elution; 60 fractions of 8 ml were collected at a rate of 3 ml/min. The polarity of the eluent was increased every 10 fractions: petrol ether/ether 1:1, petrol ether/ether 1:4, ether, ether/methanol 99:1, ether/methanol 98:2, ether/methanol 95:5, Fractions 25–36 contained metabolite **7**, fractions 37–46 metabolite **6**. All metabolites were further purified by prep. TLC. (yields see Table 1).

4. *Analytical data.* The Rf values of metabolites **1**, **2**, **4**, **5**, **6**, **7**, Δ^1-THC (**3**) and of the reference compounds 6β-hydroxy-Δ^1-THC, 7-hydroxy-Δ^1-THC and 6α,7-dihydroxy-Δ^1-THC (determined in three solvent systems and corrected to give Rf 1.00 for Δ^1-THC (**3**)) as well as the GLC. retention times of **1**, **2**, **3**, **4**, **5**, 6β-hydroxy-Δ^1-THC, 7-hydroxy-Δ^1-THC and of the trimethylsilyl ethers **5a**, **6a** and **7a** are given in Table 3.

Table 3. *Rf values and GLC. retention times*

Compound	Rf [a]	[b]	[c]	Retention time [d] (min.)
Δ^1-THC (**3**)	1.00	1.00	1.00	2.80
6β-hydroxy-Δ^1-THC	0.74	0.80	0.74	4.83
6α-hydroxy-Δ^1-THC (**4**)	0.61	0.73	0.56	4.92
3″-hydroxy-Δ^1-THC (**2**)	0.61	0.73	0.57	5.17
4″-hydroxy-Δ^1-THC (**1**)	0.55	0.68	0.52	5.10
7-hydroxy-Δ^1-THC	0.56	0.68	0.49	6.45
4″-hydroxy-6-oxo-Δ^1-THC (**5**)	0.37	0.62	0.34	9.67
Trimethylsilyl ether **5a**				7.17
4″,6α-dihydroxy-Δ^1-THC (**7**)	0.22	0.45	0.16	
Trimethylsilyl ether **7a**				7.87
4″,7-dihydroxy-Δ^1-THC (**6**)	0.11	0.32	0.09	
Trimethylsilyl ether **6a**				8.72
6α,7-dihydroxy-Δ^1-THC	0.08	0.25	0.08	

[a] Solvent system: ether.
[b] $CHCl_3$/acetone 3:2.
[c] $CHCl_3$/ethyl acetate 3:2.
[d] Retention times were determined at 230°, carrier flow 31.5 ml N_2/min, column (1.8 m × 2 mm) of 3% SE-30 on Gaschrom Q.

Mass spectra. The mass spectral data of the metabolites **1**, **2**, **4**, **5** and the trimethylsilyl ethers **1a**, **2a**, **4a**, **5a**, **6a** and **7a** are summarized in Table 2.

^1H-*NMR. spectra.* 4″-*hydroxy-*Δ^1-*THC* (**1**) (in $CDCl_3$): 1.07 (*s*, 3H, 3H–C(10)); 1.16 (*d*, $J_{4'',5''} = 6$, 3H–C(5″)); 1.41 (*s*, 3H–C(9)); 1.66 (*s*, br., 3H, 3H–C(7)); 2.44 (*t*, $J_{1'',2''} = 7$, 2H, 2H–C(1″)); 3.17 (*d*, br., $J_{3,4} = 11$, 1H, H–C(3)); 3.77 (4 lines, $J_{3'',4''} = J_{4'',5''} = 6$, 1H, H–C(4″)); 6.13 (*d*, $J_{3',5'} = 1$, 1H, H–C(3′)); 6.23 (*d*, $J = 1$, 1H, H–C(5′)); 6.30 (*s*, br., 1H, H–C(2)).

4″,7-dihydroxy-Δ^1-THC (**6**) (in $CDCl_3$): 1.09 (*s*, 3H, 3H–C(10)); 1.17 (*d*, $J_{4'',5''} = 7$, 3H, 3H–C(5″)); 1.37 (*s*, 3H, 3H–C(9)); 2.46 (*t*, $J_{1'',2''} = 6$, 2H, 2H–C(1″)); 3.24 (*d*, br., $J_{3,4} = 12$, 1H, H–C(3)); 3.78 (*m*, 1H, H–C(4″)); 4.01 (*s*, br., 2H, 2H–C(7)); 6.12 (*s*, br., 1H, H–C(3′)); 6.23 (*s*, br., 1H, H–C(5′)); 6.68 (*s*, br., 1H, H–C(2)).

4″,6α-dihydroxy-Δ^1-THC (**7**) (in $(CD_3)_2CO$): 1.07 (*s*, 3H, 3H–C(10)); 1.10 (*d*, $J_{4'',5''} = 6$, 3H, 3H–C(5″)); 1.36 (*s*, 3H, 3H–C(9)); 1.72 (*s*, 3H, 3H–C(7)); 2.42 (*t*, $J_{1'',2''} = 6$, 2H, 2H–C(1″)); 3.24 (*d*, br., $J_{3,4} = 13$, 1H, H–C(3)); 3.69 (4 lines, $J_{3'',4''} = J_{4'',5''} = 6$, 1H, H–C(4″)); 4.19 (*m*, 1H, H–C(6)); 6.10 (*s*, br., 1H, H–C(3′)); 6.25 (*s*, br., 1H, H–C(5′)); 6.56 (*s*, br., 1H, H–C(2)).

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