

## 268. Microbial Transformation of Cannabinoids. Part 3<sup>1)</sup>: Major Metabolites of (3*R*, 4*R*)- $\Delta^1$ -Tetrahydrocannabinol<sup>2)</sup>

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### Summary

The metabolic transformations of the psychotropic cannabinoid (3*R*, 4*R*)- $\Delta^1$ -tetrahydrocannabinol (**5**) (=  $\Delta^1$ -THC) by cultures of *Fusarium nivale*, *Gibberella fujikuroi* (both *Ascomycetes*) and *Thamnidium elegans* (*Phycomycetes*) were investigated. A number of metabolites, **1–4** and **6–9** were isolated from the incubations, partly purified and their structures elucidated by combined gas chromatography/mass spectrometry. Four of these metabolites, 1''-hydroxy- $\Delta^1$ -THC (**4**), 2''-hydroxy- $\Delta^1$ -THC (**1**), 6 $\beta$ -hydroxy- $\Delta^1$ -THC (**8**) and 2'', 6 $\xi$ -dihydroxy- $\Delta^1$ -THC (**9**) so far have not been reported as microbial transformation products of **5**.

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**Introduction.** – Recently *Ohlsson et al.* [3] reported the synthesis of five side chain monohydroxylated derivatives of  $\Delta^{1(6)}$ -tetrahydrocannabinol (=  $\Delta^{1(6)}$ -THC). The compounds bearing hydroxyl groups in 2'', 3'', 4''- and 5''-position were demonstrated to be psychotropically active in the rhesus monkey. So far no chemical synthesis has been published for the corresponding derivatives of (3*R*, 4*R*)- $\Delta^1$ -tetrahydrocannabinol (**5**) (=  $\Delta^1$ -THC) which constitutes the major psychotropic principle of the drug hashish,  $\Delta^{1(6)}$ -THC being an artefact of rearrangement of  $\Delta^1$ -THC (**5**) obtained upon storage of the drug. Because of the lability of the double bond located in the isoprenoid part of  $\Delta^1$ -THC (**5**) synthetic work is far more complicated than with the relatively stable  $\Delta^{1(6)}$ -THC. Due to these difficulties there has been considerable interest in the microbial transformation of cannabinoids, especially  $\Delta^1$ -THC (**5**) as a means to obtain potentially psychotropic side chain hydroxylated derivatives.

We have reported an extensive screening for microorganisms able to transform  $\Delta^1$ -THC (**5**) [1] and the microbial transformation of  $\Delta^1$ -THC by the zygomycete *Cunninghamella blakesleeana* [4] leading among other metabolites to 3''-hydroxy- $\Delta^1$ -THC (**2**) and 4''-hydroxy- $\Delta^1$ -THC (**3**), the latter being psychotropically active in the rhesus monkey [5]. 3''-Hydroxy- $\Delta^1$ -THC (**2**) was obtained in minimal yield and, therefore, could not be tested for pharmacological activity.

<sup>1)</sup> Part 2: see [1].

<sup>2)</sup> Presented in part at the 'Frühjahrstagung der GBCh, Münster, 1980' [2].

Independent of our work several studies on the microbial transformation of cannabinoids have been published. *Robertson et al.* [6-8] reported the formation of 4''-hydroxy- $\Delta^1$ -THC (3) from  $\Delta^1$ -THC (5) by *Syncephalastrum racemosum*, nitration of cannabinoids by soil bacteria and the formation of acidic cannabinoid metabolites by *Mycobacterium rhodochrous*. *Abbott et al.* [9] and *Archer et al.* [10] investigated the biotransformation of nabilone, a synthetic cannabinoid. *Vidic et al.* [11] described microbial transformation products of  $\Delta^1(6)$ -THC by *Streptomyces lavendulae* and *Pellicularia filamentosa* and *Christie et al.* [12] reported the transformation of  $\Delta^1$ -THC (5) by cultures of *Chaetomium globosum* to 3''-hydroxy- $\Delta^1$ -THC (2), 7-hydroxy- $\Delta^1$ -THC (6) and 3'',7-dihydroxy- $\Delta^1$ -THC.

As a result of our screening [1] we have defined two major types of transformation patterns of  $\Delta^1$ -THC (5) which we termed '*Fusarium type*' and '*Botrytis type*' after prominent members of two groups of microorganisms leading to the same fingerprint of metabolites as demonstrated by twodimensional thin layer chromatography (cf. [1]). Beside these major types a group of microorganisms were termed '*Miscellaneous*', each of them giving an individual pattern of cannabinoid metabolites. Since *Christie et al.* [12] have investigated the transformation of  $\Delta^1$ -THC (5) by *Chaetomium globosum*, a microorganism belonging to the '*Botrytis type*', we concentrated our efforts on two microorganisms belonging to the '*Fusarium type*', i.e. *Fusarium nivale* and *Gibberella fujikuroi* and the '*Miscellaneous*' microorganism *Thamnidium elegans*. We wish to report on the incubation of  $\Delta^1$ -THC (5) with cultures of these microorganisms, the isolation, partial purification and structure elucidation of the metabolites.

**1. Incubation of  $\Delta^1$ -THC (5).** - The microorganisms were cultivated on mycophilic agar slants. Submerge cultures were grown in cornsteep liquor medium (1 l) at 30° for four days and an additional three days at 14°, the optimal temperature for the formation of mono- and dihydroxylated metabolites of  $\Delta^1$ -THC (5) (cf. [1]). At the end of this period the pH of the cultures ranged between 7.0 and 7.5. A solution of 100 mg  $\Delta^1$ -THC (5) in 25 ml ethanol was added to the cultures and the incubation continued at 14° in the dark for 72 h.

**2. Extraction and purification of the metabolites.** - Culture media and mycelia were separated by filtration. The culture media were extracted with ethyl acetate, the mycelia with methanol. The separation of the metabolites (gradient elution on *Florisil* followed by isopolar chromatography on *Sephadex LH-20*) being a standard procedure [4] [13], experimental details will not be given here.

The yields of the metabolites were roughly estimated by gas liquid chromatography (GLC.) (peak area compared to  $\Delta^1$ -THC, see Table 1).

**3. Structures of the metabolites.** - The structures of the metabolites were elucidated by combined gas chromatography/mass spectrometry of their trimethylsilyl ethers (=TMS ethers), designated as **1a-4a** and **6a-9a**. The fragmentations of the TMS ethers of hydroxylated cannabinoids have been discussed in detail [14] [15] and a set of rules, allowing the unequivocal determination of the position of hydroxylation by specific 'lead ions' has been established [13]. The structures of the metabolites are given in the *Scheme* and the MS. data of their TMS ethers ( $M^+$  and lead ions) are summarized in Table 2.

*Fusarium nivale* and *Gibberella fujikuroi* both converted  $\Delta^1$ -THC (5) to metabolites **1**, **2** and **3**. According to the MS. of their TMS ethers metabolite **1** is 2''-hydroxy- $\Delta^1$ -THC, **2** is 3''-hydroxy- $\Delta^1$ -THC and **3** is 4''-hydroxy- $\Delta^1$ -THC. In addition to metabolites **1**, **2** and **3**, *Thamnidium elegans* formed 1''-hydroxy- $\Delta^1$ -THC (**4**), 7-hydroxy- $\Delta^1$ -THC (**6**), 6 $\alpha$ -hydroxy- $\Delta^1$ -THC (**7**), 6 $\beta$ -hydroxy- $\Delta^1$ -THC (**8**) and 2'',6 $\xi$ -dihydroxy- $\Delta^1$ -THC (**9**). 6 $\alpha$ -Hydroxy- $\Delta^1$ -THC (**7**) and 6 $\beta$ -hydroxy- $\Delta^1$ -THC (**8**), the MS. of their TMS ethers **7a** and **8a** giving the same lead ions, were

Table 1. Yield in % metabolic conversion

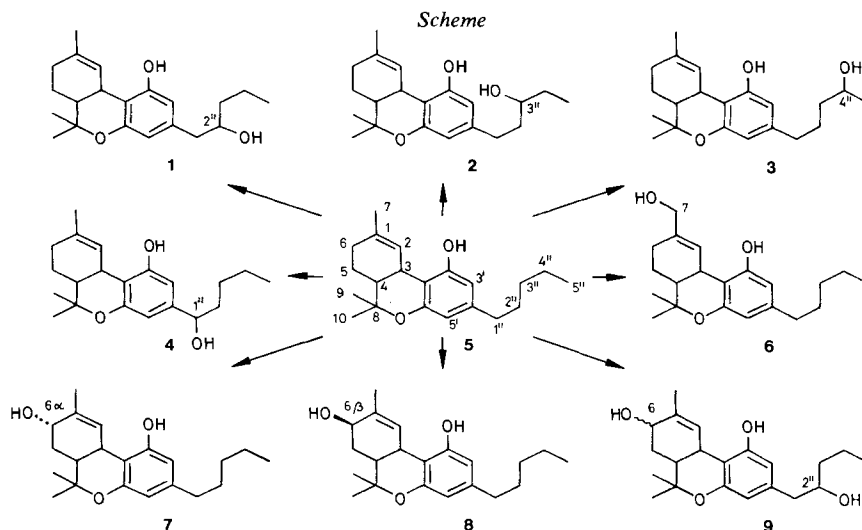
|                             | 1    | 2    | 3    | 4   | 6    | 7    | 8   | 9    |
|-----------------------------|------|------|------|-----|------|------|-----|------|
| <i>Fusarium nivale</i>      | 0.35 | 0.6  | 1.7  |     |      |      |     |      |
| <i>Gibberella fujikuroi</i> | 0.07 | 0.1  | 0.54 |     |      |      |     |      |
| <i>Thamnidium elegans</i>   | 0.24 | 0.18 | 0.03 | 0.2 | 0.06 | 0.13 | 0.2 | 0.16 |

Table 2. MS. data of the metabolites

| Metabolite                                        | $M^+$ a) (R.I.) <sup>b)</sup> | Lead ions (R.I.)                 |
|---------------------------------------------------|-------------------------------|----------------------------------|
| <b>1a</b> 2''-TMSO- $\Delta^1$ -THC               | 474 (20)                      | $m/z$ 145 (100)                  |
| <b>2a</b> 3''-TMSO- $\Delta^1$ -THC               | 474 (24)                      | $M^+$ - 144 (100)                |
| <b>3a</b> 4''-TMSO- $\Delta^1$ -THC               | 474 (100)                     | $m/z$ 117 (18)                   |
| <b>4a</b> 1''-TMSO- $\Delta^1$ -THC               | 474 (1)                       | $M^+$ - 57 (100)                 |
| <b>6a</b> 7-TMSO- $\Delta^1$ -THC                 | 474 (16)                      | $M^+$ - 103 (100)                |
| <b>7a</b> 6 $\alpha$ -TMSO- $\Delta^1$ -THC       | 474 (16)                      | $M^+$ - 90 (100)                 |
| <b>8a</b> 6 $\beta$ -TMSO- $\Delta^1$ -THC        | 474 (14)                      | $M^+$ - 90 (100)                 |
| <b>9a</b> 6 $\xi$ , 2''-bis-TMSO- $\Delta^1$ -THC | 562 (2)                       | $M^+$ - 90 (34); $m/z$ 145 (100) |

a) The molecular weight given by  $M^+$  always includes an additional TMS group at the phenolic O-atom.

b) R.I. = relative intensity in % of the base peak.



distinguished by comparison of their  $R_f$ -values with authentic samples. The configuration at C(6) in **9** could not be assigned. Metabolites **1**, **4**, **8** and **9** so far have not been described as microbial transformation products of  $\Delta^1$ -THC (**5**).

**4. Discussion.** - From these results we conclude that there are two different enzyme systems capable to hydroxylate  $\Delta^1$ -THC (**5**). System 1, which is common to the '*Fusarium type*' microorganisms is restricted in its hydroxylating capacity to the side chain C-atoms C(2''), C(3'') and C(4'') of cannabinoids. In addition to this 'Aliphatic Hydroxylase' *Thamnidium elegans* possesses an 'Allylic Hydroxylase'

Table 3. *R<sub>f</sub>* values of the metabolites

| Compound                                    | <i>R<sub>f</sub></i>               |                                          |      |
|---------------------------------------------|------------------------------------|------------------------------------------|------|
|                                             | a)                                 | b)                                       | c)   |
| $\Delta^1$ -THC (5)                         | 1.00                               | 1.00                                     | 1.00 |
| 2''-hydroxy- $\Delta^1$ -THC (1)            | 0.69                               | 0.83                                     | 0.68 |
| 3''-hydroxy- $\Delta^1$ -THC (2)            | 0.61                               | 0.73                                     | 0.57 |
| 4''-hydroxy- $\Delta^1$ -THC (3)            | 0.55                               | 0.68                                     | 0.52 |
| 1''-hydroxy- $\Delta^1$ -THC (4)            | 0.64                               | 0.85                                     | 0.64 |
| 7-hydroxy- $\Delta^1$ -THC (6)              | 0.56                               | 0.68                                     | 0.49 |
| 6 $\alpha$ -hydroxy- $\Delta^1$ -THC (7)    | 0.61                               | 0.73                                     | 0.56 |
| 6 $\beta$ -hydroxy- $\Delta^1$ -THC (8)     | 0.74                               | 0.80                                     | 0.74 |
| 2'',6 $\xi$ -dihydroxy- $\Delta^1$ -THC (9) | 0.52                               | 0.47                                     | 0.36 |
| a) Solvent system: ether.                   | b) CHCl <sub>3</sub> /acetone 3:2. | c) CHCl <sub>3</sub> /ethyl acetate 3:2. |      |

capable to hydroxylate  $\Delta^1$ -THC (5) in positions 1'', 6 and 7. The occurrence of metabolite 9, 2'',6 $\xi$ -dihydroxy- $\Delta^1$ -THC demonstrates that monohydroxylated metabolites can serve as substrates for the second enzyme system.

The low yields of metabolites did not allow any pharmacological evaluation of activity and seem to exclude microbiological transformation of cannabinoids as a preparative method for obtaining side chain hydroxylated derivatives of  $\Delta^1$ -THC (5).

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#### Experimental Part

*General remarks* see [4] [13].

*Culture media and incubations:* see [1].

*Separation of the metabolites:* see [4] [13].

*Analytical data.* The *R<sub>f</sub>*-values of metabolites 1–4 and 6–9 and  $\Delta^1$ -THC (5), corrected to *R<sub>f</sub>* ( $\Delta^1$ -THC (5)) = 1.00 in three solvent systems are given in Table 3.

MS. (*M*<sup>+</sup> and lead ions) of 1a–4a and 6a–9a see Table 2.

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