Toxichem Krimtech 2011;78(Special Issue):316

Regioselective synthesis of THCA-A and THCA-B by reaction of Δ9-THC with magnesium methyl carbonate (MMC)

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Key words: Δ9-Tetrahydrocannabinolic acid A, Δ9-Tetrahydrocannabinol, THCA-A, THCA-B, cannabis consumption marker, regioselective synthesis

Abstract

Aim: Δ9-Tetrahydrocannabinolic acid A (THCA-A) is the non psychoactive precursor of Δ9-Tetrahydrocannabinol (Δ9-THC) and the main cannabinoid component in fresh hemp material. During the smoking process, only a part of THCA-A is converted into the psychoactive Δ9-THC. Therefore THCA-A is a promising candidate for use as a consumption marker. For reliable quantification of THCA-A by LC-MS/MS or GC-MS/MS, an isotopically labelled internal standard is required. The presented synthesis strategy is based on a paper of Mechoulam et al. from 1969. The carboxylation process as described in this paper, yields a mixture of product (THCA-A) and 82 % unchanged starting material (Δ9-THC). Conditions of synthesis were varied systematically to optimize yield and purity of product.

Methods: Before using the rather expensive isotopically labelled Δ9-THC-D_3, conditions of synthesis were evaluated using purified Δ9-THC, derived from fresh plant material. Δ9-THC was heated in a 2M-solution of MMC in DMF at 120°C for 3 h. After hydrolysis with diluted HCl and ether extraction the products were characterized by GC-MS.

Results: Dependent on the reaction conditions different products could be obtained.
Discussion: Using only a few equivalents of MMC and hydrolysis at RT proved to suit best for synthesis of THCA-A, concerning yield as well as isomer purity. With a higher excess of reagent and hydrolysis at low temperatures, the mass spectrum of the final product indicates the formation of Δ9-Tetrahydrocannabinolic acid B (THCA-B).

Conclusion: We investigated the impact of synthesis conditions on carboxylation of Δ9-THC by use of various amounts of MMC and different temperatures for hydrolysis. A novel approach for the regioselective synthesis of THCA-B was discovered.

1. Introduction

Cannabis is still the most commonly used illegal drug in Germany [1]. For proof of cannabis use, forensic laboratories analyze blood, urine and hair samples for Δ9-THC and its oxidative metabolites. THCA-A is currently not covered in routine analysis.

THCA-A is the non psychoactive precursor of Δ9-THC and the main cannabinoid component in fresh hemp material. While smoking a joint or baking cookies, THCA-A is partially converted into the psychoactive Δ9-THC via decarboxylation. Therefore THCA-A is incorporated to a certain extent by cannabis consumers and is a promising candidate for use as an additional consumption marker.

For reliable quantification of THCA-A by LC-MS/MS or GC-MS/MS, an isotopically labelled internal standard is required. By now such an internal standard is not commercially available. Hence, a strategy for synthesis of such a standard was developed. A carboxylation procedure published by Mechoulam et al. for the synthesis of different cannabinolic acids

using MMC as carboxylating agent [2] was evaluated. Before using the rather expensive isotopically labelled Δ9-THC-D_3 as starting material, conditions of synthesis were evaluated with purified Δ9-THC derived from plant material. For optimisation we focused on variable amounts of MMC (10-250 molar equivalents) and different temperatures for hydrolysis (RT, 4°C).

2. Materials and Methods

2.1. Synthesis

To evaluate synthesis conditions we used Δ9-THC derived from cannabis raw extracts after purification by flash chromatography. A 2M-solution of MMC in DMF as well as methyl *tert*-butylether and hydrochloric acid (fuming 37 %, ACS) were purchased from Sigma Aldrich (Steinheim, Germany). Methanol (HPLC-gradient grade) was bought from J.T. Baker (Deventer, The Netherlands), Na_2SO_4 (p. a.) was purchased from Merck (Darmstadt, Germany).
General synthesis conditions: purified Δ9-THC was heated in a 2M-solution of MMC in DMF at 120°C for 3 h [2]. After hydrolysis with diluted HCl and extraction with methyl *tert*-butyl-ether, reaction products were identified using GC-MS after derivatisation. The following parameters were varied during synthesis: amount of MMC (10-250 molar equivalents) and hydrolysis temperature (RT approx. 20°C, on ice approx. 4°C).

2.2. Identification

For identification of products 50 µL of the methanolic solution were evaporated under a stream of nitrogen at room temperature. For derivatisation the residue was mixed with 25 µL N-methyl-N-(trimethylsilyl)-trifluoracetamid (MSTFA) as well as 25 µL ethyl acetate and heated for 45 min at 90°C. MSTFA was purchased from Macherey-Nagel (Düren, Germany) and ethyl acetate (ACS grade) from Sigma Aldrich (Steinheim, Germany).

1 µL was injected in the GC-MS system (Agilent Technologies, Waldbronn, Germany; gas chromatograph 6890N, MSD 5973N). GC-MS conditions were as follows: splitless injection, capillary column (HP-5MS: 30m x 0,25 mm x 0,25 µm), injection port temperature: 250°C, carrier gas: helium with a flow rate of 1,4 mL/min, oven temperature program: 140°C for 2 min, increased to 200°C at 60°C/min, to 230°C at 2,5°C/min and to 310°C at 60°C/min (hold 3 min). Total run-time was 19.3 min.

Detection was performed by a mass selective detector in full-scan mode after electron impact ionization (EI): ionization energy: 70 eV, m/z 50 – 550 amu; ion source temperature: 230 °C.

2.3. Purification

For purification of synthesis products and separation of unchanged starting material, preparative HPLC was used (column: Synergi 10 µm Hydro-RP 80A, 250 x 15 mm, purchased from Phenomenex, Aschaffenburg, Germany). The mobile phase composition was as follows: A= HCOOH pH 2.3 (10 %) and B = MeOH (90 %), flow rate: 25 mL/min. Formic acid for dilution of HCOOH (pH 2,3) was purchased from Carl Roth (Karlsruhe, Germany).

3. Results and Discussion

Using 10 molar equivalents of MMC and hydrolysis at RT proved to suit best for synthesis of THCA-A, concerning yield (approx. 13 % referring to the applied amount of Δ9-THC) as well as isomer purity.

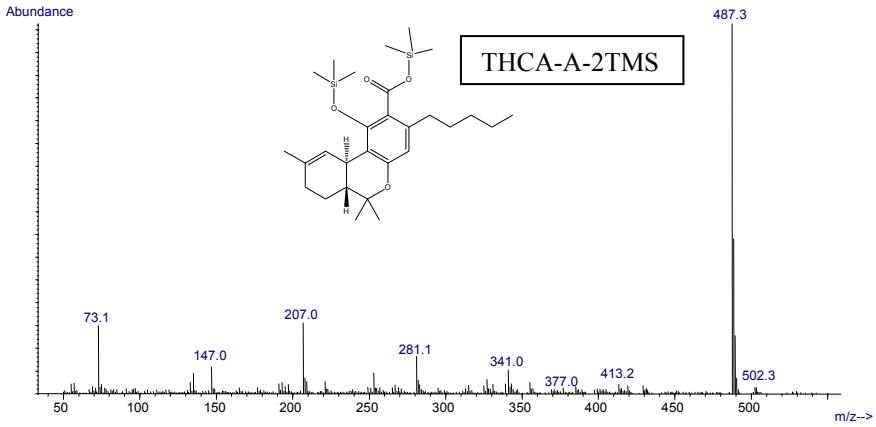

Fig. 1. EI-MS spectrum of THCA-A-2TMS.

The highly intense fragment 487 (m/z) is the result of the elimination of a methyl group from the phenolic TMS moiety, yielding a stable cyclic fragment ion [3].

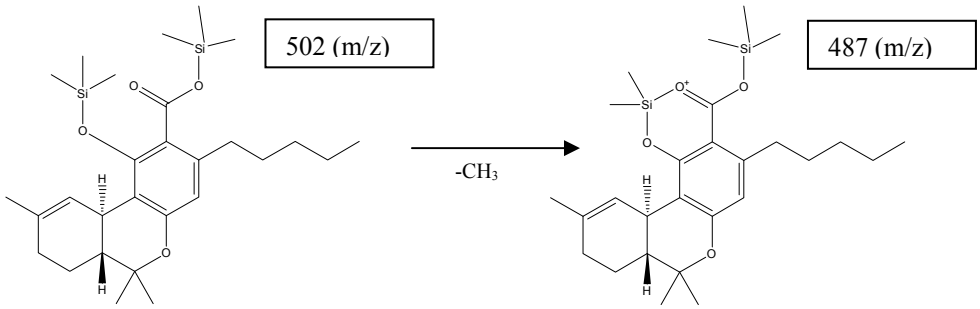

Fig. 2. Formation of the cyclic fragment ion 487 (m/z).

With a higher excess of MMC (250-fold) and hydrolysis on ice, the mass spectrum of the final product indicates the formation of Δ9-Tetrahydrocannabinolic acid B (THCA-B). This is shown by an inversion of the intensities of the typical ion fragments 487 m/z and 502 m/z in the GC-EI-MS spectrum [4].

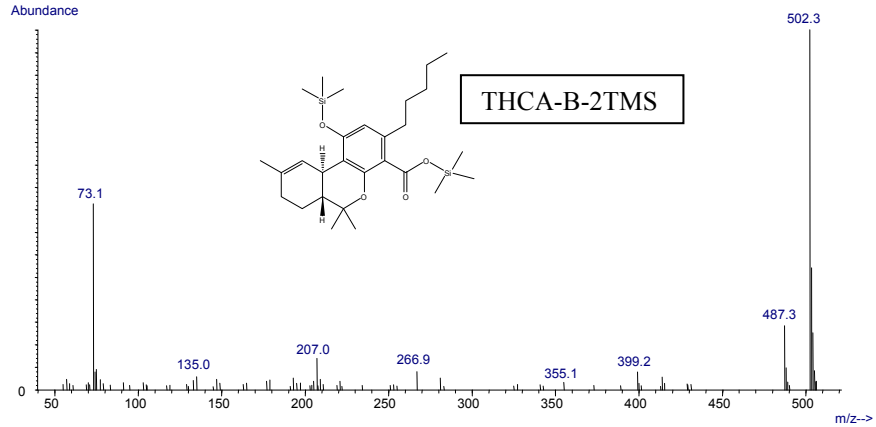

Fig. 3. EI-MS spectrum of THCA-B-2TMS.

Due to the unfavourable position of the carboxyl-group, the formation of a stable cyclic fragment is improbable for THCA-B [3]. Therefore the molecular ion 502 (m/z) is dominating.
With an extremely high excess of MMC (>250-fold) and hydrolysis on ice, mixtures of both products were obtained (between 7 % THCA-A and 9 % THCA-B yield).
Furthermore the influence of moisture to the reagent was investigated. In general moisture should be strictly avoided to prevent hydrolysis of MMC. Adding only 5 µL water to the reagent decreased yield of THCA-A and THCA-B to zero.

4. Conclusions

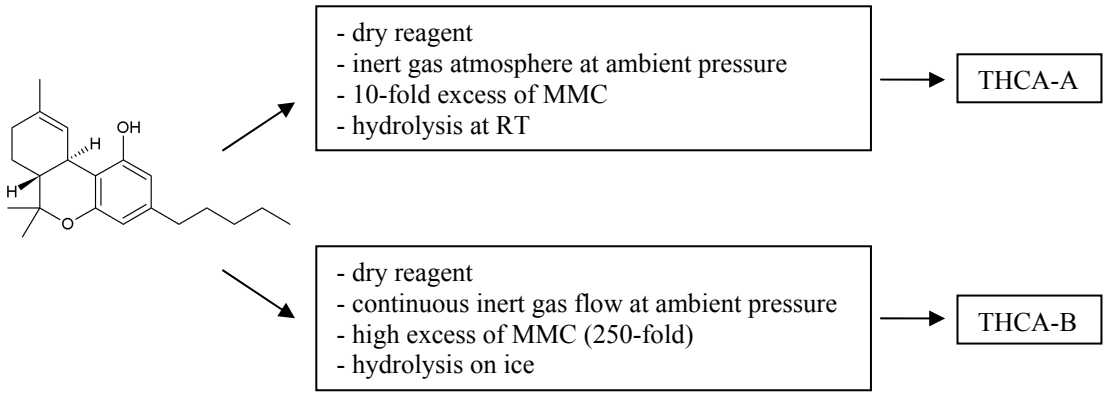

Fig. 4. Optimized conditions for synthesis of THCA-A and THCA-B with MMC.

Optimized conditions for the successful synthesis of THCA-A were found as indicated in Fig. 4. The conversion of Δ9-THC-D_3 to the corresponding Δ9-THCA-A-D_3 is expected to proceed in analogy to the presented synthesis. Furthermore, we found a way to synthesize the natural cannabinoid THCA-B. However, since only traces of THCA-B are present in cannabis material, THCA-B – in contrast to THCA-A – seems to be less suitable as a marker for cannabis use.

The authors would like to thank Prof. Dr. Michael Müller (Institute of Pharmaceutical and Medical Chemistry, University of Freiburg) for helpful discussions.

5. References

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