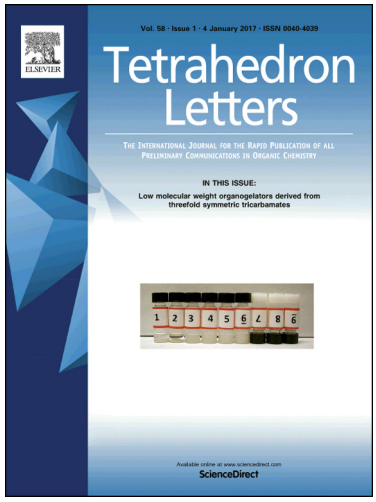

Synthesis and identification of an important metabolite of AKB-48 with a secondary hydroxyl group on the adamantyl ring

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Synthesis and identification of an important metabolite of AKB-48 with a secondary hydroxyl group on the adamantyl ring

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ABSTRACT

Studies on the metabolism of bioactive substances containing the adamantyl moiety have shown that hydroxylation is likely to occur at a tertiary carbon of adamantane. Herein, we report the synthesis and identification of one major metabolite of AKB-48, a new illicit psychoactive substance with a hydroxyl group at a secondary carbon of the adamantyl ring.

Introduction

Adamantane is an important moiety in many bioactive substances with a broad spectrum of biological activities.[1] Current approved therapeutic agents containing the adamantyl moiety range from remedies for *Influenza A, Herpes simplex* and *Acne vulgaris* to treatments for Parkinsonism, Alzheimer's disease and type II diabetes mellitus (Vildaglitin, Saxagliptin). Adamantane is also a common building block in new psychoactive substances (NPS), for example AKB-48, 5F-AKB-48, APICA and AB001.[2]

Investigations regarding the metabolism of bioactive substances containing the adamantyl moiety have been extensively carried out.[3] Interestingly, most reports show that the tertiary carbons of the adamantyl moiety are much more prone to oxidation than the secondary carbons unless more than two tertiary carbons are already substituted. For example, 1-amino-3-hydroxyadamantane was the only detected metabolite of amantadine.[4] To the best of our knowledge, there are no reports regarding adamantane containing bioactive substances that exclusively produce a metabolite with a hydroxyl group on a secondary carbon of the adamantyl moiety.

AKB-48, which contains the adamantane moiety, belongs to a family of synthetic cannabinoids that has gained increased popularity as a substitute to cannabis abuse in recent years. Challenges in detecting synthetic cannabinoids are largely due to their fast *in vivo* metabolism, together with a lack of knowledge concerning their metabolism. The limited access to reference substances and optimized detection methods for these substances in urine are also of concern. Several studies have been conducted on the metabolism of AKB-48 using HPLC-QTOF-MS to analyse the culture media of human liver microsomes or hepatocytes. The metabolite with a single hydroxyl group on the adamantyl moiety was found to be a major metabolite with an ambiguous structure (Fig. 1),[5] making it a good candidate for use as a biomarker for AKB-48 intake. This necessitated identification of the exact position of the hydroxyl group, requiring the synthesis of relevant reference substances. Although isolation and identification of metabolites present in urine samples would also work, such an approach is less appropriate as it requires large quantities of samples and extensive animal experiments. [3] This makes the synthesis of metabolites more applicable in the battle against the rapidly growing illegal drug market.

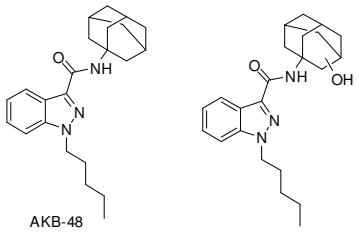

Figure 1. AKB-48 and its major metabolite with a single hydroxyl group on the adamantyl moiety.

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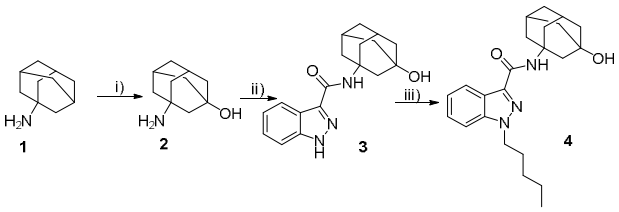
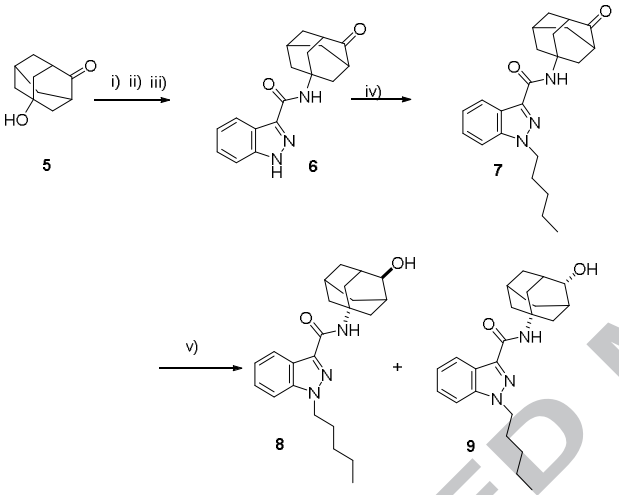
Results and discussion

Adamantine **1** was oxidized using concentrated H_2SO_4 and HNO_3 at 10 °C to give 3-hydroxy-adamantine **2** in 74% yield,[6] which was coupled with 3-indazole-carboxylic acid to selectively form amide **3**. Compound **4** was obtained after the alkylation of **3** with bromopentane in an overall yield of 54% (Scheme 1).



Scheme 1. i) conc. H_2SO_4, conc. HNO_3, 2 h, 10 °C, 74%; ii) TBTU, 1*H*-indazole-3-carboxylic acid, Et_3N, THF, rt, overnight, 81%; iii) 1-bromopentane, *t*-BuOK, THF/DMF (5:1), rt overnight, 90%.



Scheme 2. i) CH_3CN, $BF_3{\cdot}Et_2O$, TFA; ii) conc. HCl (aq), 150 °C MW irradiation, 1 h; iii) TBTU, 1*H*-indazole-3-carboxylic acid, Et_3N, THF, rt, overnight, 30% over three steps; iv) 1-bromopentane, *t*-BuOK, DMF/THF (1:5), rt, overnight, 72%; v) $NaBH_4$, **8**, 36% and **9**, 20%.

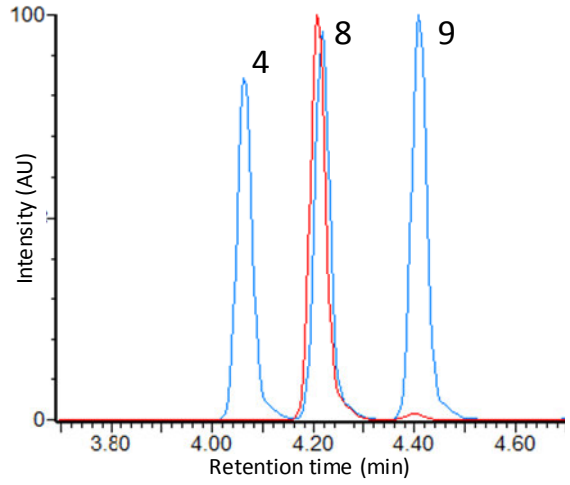


Figure 2. Analysis of reference materials from compounds **4**, **8** and **9** (blue trace), and a urine sample (red trace) by LC-MS.

According to the literature method,[7] the hydroxyl group of compound **5** was converted into an amine group *via* a Ritter reaction, followed by hydrolysis with HCl (aq) under microwave irradiation. The resulting crude product was coupled with 3-indazole-carboxylic acid to give compound **6**. This ketone was alkylated and reduced to give two isomers, **8** and **9**, in an overall yield of ~8% and 4%, respectively, over five steps (Scheme 2). The yield of the final step was low due to the difficulty of separating the two isomers. Their structures were elucidated using 1D and 2D NMR (ESI).

The NPS are generally screened using LC-HRMS in forensic toxicology laboratories. During routine LC methods applied at the National Board of Forensic Medicine (RMV), the three potential metabolites **4**, **8** and **9** co-eluted. A structured method development process was successfully applied to establish an LC method, enabling the separation of these compounds. In this method, an LC gradient from 26 to 67% MeCN in 10 mM ammonium acetate on a 100x2.1 mm Cortecs UPLC C18 column (Waters) over 7.5 min was applied. Using this method, compound **8** was found to be the target metabolite (Fig. 2), which can be used as a biomarker for detection of AKB-48 in urine sample.

Conclusion

In conclusion, we report the straightforward synthesis and identification of an important metabolite of AKB-48 with a single hydroxyl group on a secondary carbon of the adamantyl moiety. The presence of this metabolite in urine samples could therefore act as a biomarker to potentially reveal the abuse of AKB-48. The study also indicates another important, more general, metabolic picture of the adamantyl moiety, namely exclusive metabolism of the adamantyl moiety on a secondary carbon. Furthermore, these results highlight the importance of having access to all the potential metabolites. Without such a set of reference substances, it could be difficult to obtain accurate metabolism results of bioactive molecules containing the adamantyl moiety.

Acknowledgments

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Supplementary Material

Supplementary data associated with this article can be found, in the online version, at

Graphical Abstract

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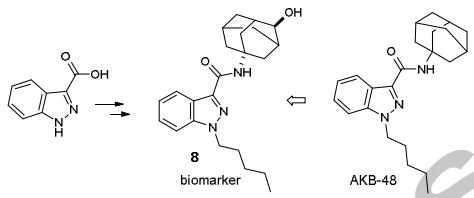

Highlights:

- Straightforward synthesis and identification of an important metabolite biomarker of AKB-48.
- Indicates another metabolic picture of adamantane exclusively with a secondary hydroxyl group.
- Highlights the importance of access to all potential metabolites for accurate metabolic study.