ChemComm

A six-step total synthesis of α-thujone and d6-α-thujone, enabling facile access to isotopically labelled metabolites

Journal:	*ChemComm*
Manuscript ID	CC-COM-06-2016-005376.R1
Article Type:	Communication
Date Submitted by the Author:	01-Sep-2016
Complete List of Authors:	Thamm, Irene; Technische Universitat Munchen, Analytical Food Chemistry Richers, Johannes; Technische Universitat Munchen, Department Chemistry Rychlik, Michael; Technische Universitat Munchen, Chair of Analytical Food Chemistry Tiefenbacher, Konrad; Universitat Basel Departement Chemie

Chem Comm

COMMUNICATION

A six-step total synthesis of α-thujone and d_6-α-thujone, enabling facile access to isotopically labelled metabolites

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Irene Thamm,[a] Johannes M. Richers,[b] Michael Rychlik,[a] Konrad Tiefenbacher*[c,d]

This short synthesis of α-thujone relies on the functionalization of the readily available dimethylfulvene. Furthermore, the three main metabolites of the natural product were also synthesized. Since d_6-acetone can be used as starting material, the route developed allows for the facile incorporation of isotopic labels which are required for detecting and quantifying trace amounts *via* GC/MS analysis.

A great variety of plants produces the monoterpenes α-thujone (**1a**) and β-thujone (**2**, Fig. 1), which therefore are present in diverse herbal products.[1] The most famous product containing thujone is certainly absinth, produced from wormwood. It had been a popular spirit drink in the 19th century but later was prohibited due to concerns about its toxicity.[2] It was connected to severe health problems, including hallucinations, depressions, convulsions, blindness and mental deterioration. More recent studies propose that most of these effects were caused by alcohol intoxication.[2] Nevertheless, thujone is neurotoxic and was shown to inhibit the gamma-aminobutyric acid A (GABAA) receptor, which leads to excitations and convulsions at higher concentrations in animal studies.[3] α-Thujone (**1a**) was shown to be more active than its β-isomer **2**. The metabolism of thujone was investigated both in-vitro and in-vivo. 7-OH-α-thujone (**3a**) is clearly the major metabolite in in-vitro studies.[3] In-vivo studies, however, point to 2-OH-α-thujone (**4a**) and 4-OH-α-thujone (**5a**) as the main metabolites.[4]

The manufacture of thujone containing products is permitted again in the European Union but maximum limits have been imposed.[5] To ensure accurate quantitation, to assess whether these products meet the requirements and additionally to better detect trace amounts of α-thujone and its major metabolites, access to isotopically labelled derivatives is required. Although the structure of the bicyclic monoterpene was elucidated already in 1900 by Semmler,[6] only one total synthesis has been reported so far.[7] Oppolzer et al. prepared enantioenriched α-thujone over twelve steps from commercially available material, utilizing an elegant palladium-catalyzed cyclization strategy. The route, however, does not allow for a facile introduction of inexpensive isotopic labels. Therefore, we developed and herein describe a novel six-step access to α-thujone, which enables the introduction of isotopic labels from inexpensive d_6-acetone. The synthesized d_6-thujone **1b** thereafter is also functionalized to the most important metabolites **3b**, **4b** and **5b**.

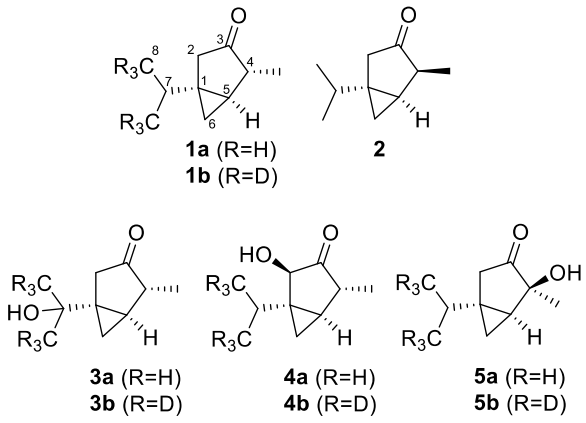
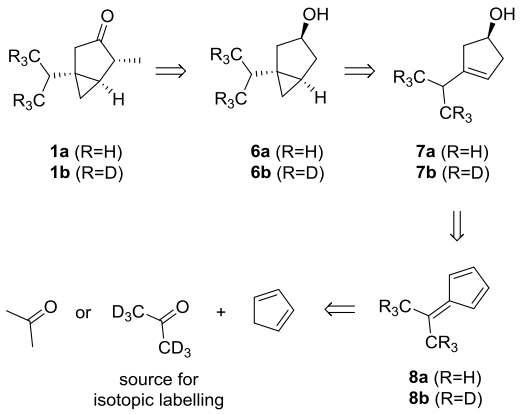


Fig. 1 Structures of α-thujone (**1a**), β-thujone (**2**) and the major metabolites of α-thujone: 7-OH-α-thujone (**3a**), 2-OH-α-thujone (**4a**), and 4-OH-α-thujone (**5a**).

Our synthetic strategy is based on a late-stage oxidation of alcohol **6a**/**6b** (Scheme 1), followed by a regio- and diastereoselective methylation at position C4. Such an approach seemed attractive since alcohol **6a**/**6b** should be directly accessible from cyclopentenol **7a**/**7b** *via* Simmons-Smith cyclopropanation.[8] Cyclopentenol **7a**/**7b** can be traced

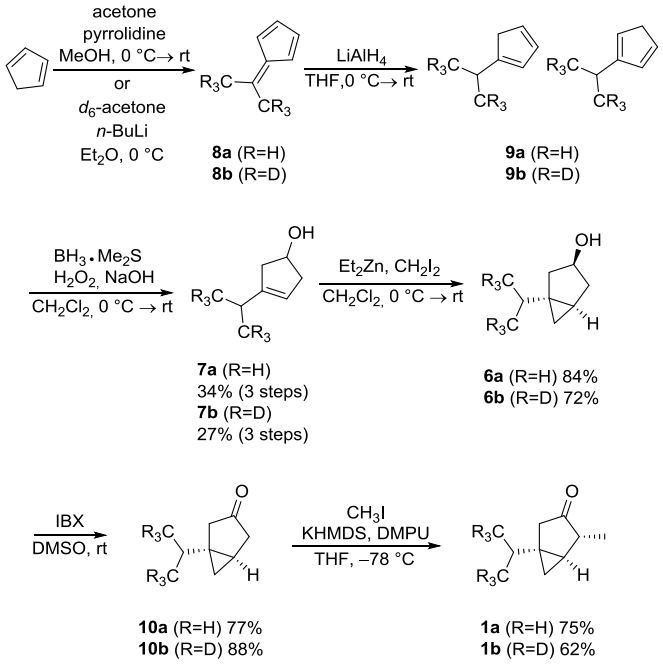
*Corresponding author
[a] Analytical Food Chemistry, Technische Universität München, Alte Akademie 10, 85354 Freising, Germany.
[b] Department of Chemistry, Technische Universität München, Lichtenbergstraße 4, 85747 Garching, Germany.
[c] Department of Chemistry, University of Basel, St. Johannsring 19, CH-4056 Basel, Switzerland. E-mail: konrad.tiefenbacher@unibas.ch
[d] Department of Biosystems Science and Engineering, ETH Zürich, Mattenstrasse 26, CH-4058 Basel, Switzerland. E-mail: tkonrad@ethz.ch
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

back to the known dimethyl fulvenes **8a** and **8b**, which are synthesized from cyclopentadiene and acetone.[9] Therefore, the inexpensive d_6-acetone can function as the source of the isotopic labels.

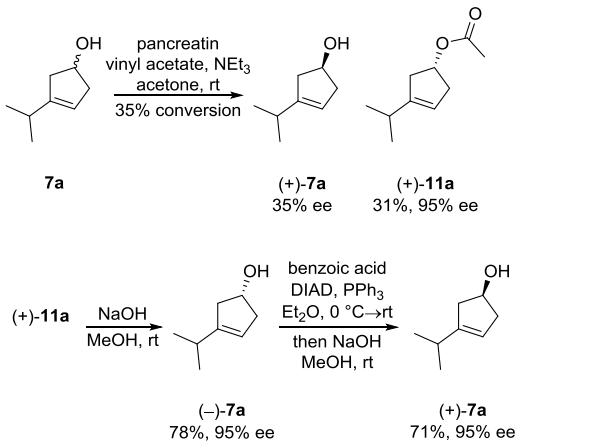


Scheme 1 Retrosynthetic analysis of ***α***-thujone (**1a**) and its deuterated derivative **1b**.

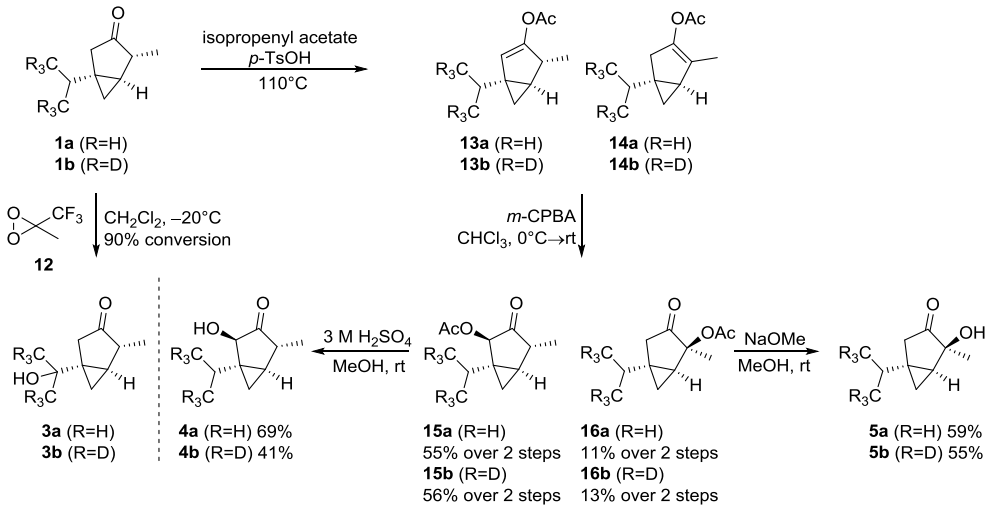
Our synthesis commenced with the formation of dimethylfulvene (Scheme 2). The preparation of **8a** followed a procedure described by Little et al., utilizing pyrrolidine as a base.[9a] This procedure was not suitable for the preparation of the d_6-dimethylfulvene **8b**, since the deuterium label was partially removed, presumably *via* enamine formation. Therefore, the procedure introduced by the group of Gajewski was utilized to prepare d_6-dimethylfulvene **8b**.[9b] Using *n*-butyllithium as base, a deuteration degree of 95.5% was achieved. The dimethylfulvenes formed were reduced with lithium aluminium hydride to yield a mixture of cyclopentadienes. It is known that related mixtures can be converted convergently to a single alcohol product *via* the hydroboration/oxidation sequence.[10] Indeed, such a procedure yielded, after purification by chromatography, alcohols **7a** and **7b** in 34% and 27% yield over three steps, respectively. Cyclopropanation was performed utilizing the Furukawa modification of the Simmons-Smith reaction.[11] After oxidation utilizing 2-iodoxybenzoic acid (IBX) as oxidant,[12] ketones **10a**/**10b** were obtained in 77% and 88% yield, respectively. The final alkylation step required careful optimization of the reaction conditions. It was found that multi-alkylated products cannot be separated by flash chromatography. Therefore, reaction conditions were optimized to produce **1a/1b** selectively. The use of 1.0 eq. of potassium bis(trimethylsilyl)amide and methyl iodide, as well as the addition of N,N'-dimethylpropyleneurea facilitated this task. Thujones **1a**/**1b** were obtained in 75% and 62% yield, respectively.



Scheme 2 Synthesis of thujone **1a**/**1b**.



Scheme 3 Kinetic resolution of (±)-3-isopropylcyclopent-3-en-1-ol and inversion of the chiral center *via* Mitsunobu reaction.



Scheme 4 Synthesis of the hydroxylated main metabolites.

Although optically active material is not required for the use as internal standard in GC/MS or LC/MS analysis, it may be helpful for other applications. Therefore, we investigated the possibility to perform the hydroboration of the cyclopentadienes **9a** in an enantioselective fashion. Attempts with diisopinocampheylborane[13] only lead to very low levels of enantiomeric enrichment (11% ee). It was therefore decided to turn to kinetic resolution. Pancreatin was utilized for the kinetic resolution of a structurally related cyclopentenol[14] and also proved effective in this case. Kinetic resolution provided acetate (+)-**11a** in high optical purity (95% ee) and acceptable conversion 35% (Scheme 3). Alcohol (+)-**7a**, the enantiomer required, was not accessible efficiently *via* kinetic resolution. Time-consuming repetitions of kinetic resolutions were required to increase the conversions and enantiomeric excess. It turned out to be advantageous to work with acetate (+)-**11** and invert its stereocenter *via* the Mitsunobu reaction. This three step procedure delivered (+)-**7a** in high optical purity (95% ee). This material was converted to optically active thujone (–)-**1a** (95% ee) as described in Scheme 2 (see SI).

After having developed a concise route to thujone (**1a**) and its isotopically labelled derivative **1b**, we turned to the preparation of the most important metabolites. The oxidation of **1a** to 7-OH-α-thujone (**3a**) was described in literature utilizing ozone as oxidant.[15] However, these conditions lead to considerable overoxidation and a reduced yield of **3a** (47%). After screening several oxidants, we found that methyl(trifluoromethyl)dioxirane[16] (TFDO, **12**) led to a clean conversion to **3a** (Scheme 4). For the synthesis of the 4-hydroxy derivative **5a**, we followed the procedure of the group of Casida.[17] First, the enolacetate is formed by refluxing thujone in isopropenyl acetate under acidic conditions. In contrast to literature, we observed the 2, 3-enol acetate **13a** as main product (**13a** : **14a** = 8:2). Therefore, we decided to utilize this mixture to obtain both, the 2-hydroxy and the 4-hydroxy derivative at once. The inseparable mixture was oxidized with 3-chloroperoxybenzoic acid. In both cases epoxidation was preferred from the convex bottom face, delivering after migration of the acetate group the desired diastereoisomers **15** and **16**. The isomers were separated by chromatography, and subsequently deprotected to yield the metabolites **4** and **5**, respectively. Deprotection of **15** was only successful under acidic conditions, as regular basic hydrolysis led to decomposition of the material.

In summary, we have developed a concise route to α-thujone, which relies on the functionalization of dimethylfulvene. The synthesis allows for the facile incorporation of inexpensive isotopic labels by utilizing d_6-acetone as starting material. Furthermore, the three main metabolites of α-thujone were prepared.

Notes and references

1 O. Pelkonen, K. Abass, J. Wiesner, *Regul. Toxicol. Pharm.* 2013, **65**, 100.
2 D. W. Lachenmeier, J. Emmert, T. Kuballa, G. Sartor, *Forensic Sci. Int.* 2006, ***158***, 1.
3 K. M. Hold, N. S. Sirisoma, T. Ikeda, T. Narahashi, J. E. Casida, *Proc. Natl. Acad. Sci. U.S.A.* 2000, **97**, 3826.
4 K. M. Höld, N. S. Sirisoma, J. E. Casida, *Chem. Res. Toxicol.* 2001, **14**, 589-595.
5 European Council Directive No. 88/388/EEC, 22 June 1988 http://ec.europa.eu/food/fs/sfp/addit_flavor/flav09_en.pdf (accessed on 23.11.2015)
6 F. W. Semmler, *Berichte der deutschen chemischen Gesellschaft* 1900, **33**, 275-277.
7 W. Oppolzer, A. Pimm, B. Stammen, W. E. Hume, *Helv. Chim. Acta* 1997, **80**, 623.
8 (a) H. E. Simmons, R. D. Smith, *J. Am. Chem. Soc.* 1958, **80**, 5323; (b) H. Lebel, J.-F. Marcoux, C. Molinaro, A. B. Charette, *Chem. Rev.* 2003, **103**, 977.
9 (a) K. J. Stone, R. D. Little, *J. Org. Chem.* 1984, **49**, 1849; (b) J. J. Gajewski, G. C. Paul, M. J. Chang, A. M. Gortva, *J. Am. Chem. Soc.* 1994, **116**, 5150.
10 S. Collins, Y. Hong, M. Kataoka, L. Nguyen The, *J. Org. Chem.* 1990, **55**, 3395.

11 J. Furukawa, N. Kawabata, J. Nishimura, *Tetrahedron Lett.* 1966, **7**, 3353.
12 M. Frigerio, M. Santagostino, *Tetrahedron Lett.* 1994, **35**, 8019.
13 H. C. Brown, B. Singaram, *J. Org. Chem.* 1984, **49**, 945.
14 (a) M. Mahler, B. Reichardt, P. Hartjen, J. van Lunzen, C. Meier, *Chem.–Eur. J.* 2012, **18**, 11046; (b) O. R. Ludek, C. Meier, *Synthesis* 2003, **13**, 2101.
15 J. P. Kutney, K. Piotrowska, Y.-H. Chen, K.-P. N. Cheng, Z. Gao, S. J. Rettig, *Can. J. Chem.* 1990, **68**, 1698.
16 R. Mello, M. Fiorentino, C. Fusco, R. Curci, *J. Am. Chem. Soc.* 1989, **111**, 6749.
17 N. S. Sirisoma, K. M. Höld, J. E. Casida, *J. Agric. Food. Chem.* 2001, **49**, 1915.

