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First synthesis of (+)-myrrhanol C, an anti-prostate cancer lead†

Cite this: *Org. Biomol. Chem.*, 2013, **11**, 559

Received 4th October 2012,
Accepted 2nd November 2012

DOI: 10.1039/c2ob26947c

www.rsc.org/obc

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Published on 02 November 2012 on http://pubs.rsc.org | doi:10.1039/C2OB26947C
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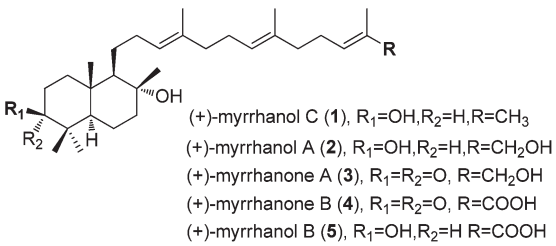
The first synthesis of (+)-myrrhanol C (1), an antitumor polypodane-type bicyclic triterpene with inhibitory activity against androgen insensitive prostate cancers, is reported herein (IC_{50} 10 μmolar). A key step in our convergent synthesis of (+)-myrrhanol C and related analogues is the employment of a microbial stereo- and regioselective late stage C–H oxidation. A low-waste and sustainable process has been developed to prepare (+)-myrrhanol C for further biological studies.

Myrrhanol C (**1**) is a natural product that was isolated from *Pistacia lentiscus*[1a] oleogum resin and the gum resin from *Commiphora mukul.*[2] Very recently, in 2011, Simmet *et al.* reported that myrrhanol C (**1**) triggers apoptosis in chemoresistant, PC-3 androgen-independent human prostate cancer cells *in vitro* and *in vivo*.[3] Although **1** may serve as a lead compound for targeting androgen-insensitive prostate cancers, no synthetic strategies for its production have been reported to date. Prostate cancer, with an estimated number of 241 740 new cases expected in the USA in 2012 (29% of all cancer cases), is the most common type of cancer after skin cancer, and also represents the second most lethal cancer.[4]

With these data in mind and continuing with our program on the synthesis of "unusually cyclized triterpenes"[5] we report herein a concise and efficient synthesis of myrrhanol C (**1**). Moreover, its synthetic construction could permit easy access to the corresponding more oxidized anti-inflammatory members of the same family of natural products **2–5**[6] which in our opinion are likely to share the anti-tumoral activity of **1**.

The biosynthetic formation of the bicyclic polypodanes with an oxygenated function at C3 should involve cyclization by oxidosqualene cyclase (OS). The acyclic precursor is postulated to adopt a chair–chair conformation which upon cyclization would result in a bicyclic carbocationic intermediate.



Scheme 1 Proposed biosynthesis of myrrhanols **1–5**.

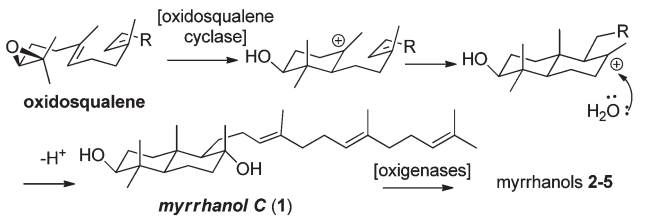


Fig. 1 Myrrhanols isolated in Nature.

Then, hydration and in some cases oxidations would lead to all the known myrrhanols **1–5** (Scheme 1) (Fig. 1).

It should be noted that a viable strategy for this family of compounds could be the one developed previously by our research group using as a key step a Ti(III)-mediated radical carbocyclization in the synthesis of myrrhanol A (**2**).[5c] However, the promising anti-cancer properties of **1** encouraged us to design a more sustainable and green biocatalytic strategy[7] for future scalable purposes.[8] The retrosynthetic analysis is shown in Scheme 2. The target molecule **1** would be obtained in a convergent approach involving the coupling between a bicyclic homodrimanic synthon **11** and the acyclic vinyl iodide **12**.

On the other hand, synthetic chemists are currently immersed in the development of methods for direct C–H functionalization, including C–H oxidation.[9] In this context, the application of this methodology in the synthesis of natural products has been reported by some authors,[10] however,

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†Electronic supplementary information (ESI) available: Experimental procedures, spectral and analytical data, as well as comparison between natural (+)-myrrhanol C (**1**) and synthetic material. See DOI: 10.1039/c2ob26947c

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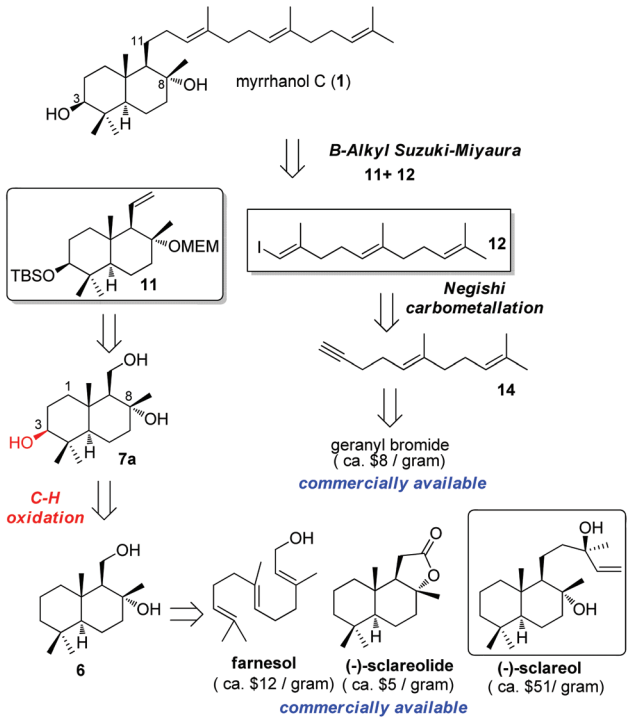
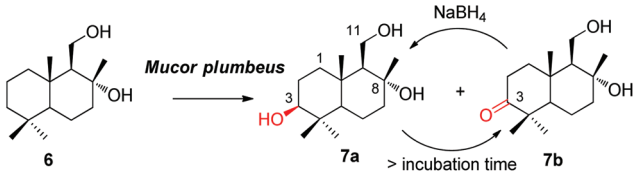


Scheme 2 Retrosynthetic analysis.

not very many reactions of this kind have been extensively exploited in the field of terpenoids. Factors such as the multitude of C–H positions, the competitive transformations between axial and equatorial positions, strain release,[11] as well as the shortage of methods available in the emerging alkane activation field are considered as some of the drawbacks for achieving selectivity using this methodology. In this context, P450 microbial strains have proven to be an attractive alternative for the selective oxidation of C–H bonds.[12]

Our synthetic strategy commenced with the preparation of the bicyclic fragment **6**. According to the literature, this compound can be easily prepared from abundant, renewable and commercially available starting materials, namely, (−)-sclareol (70% over 3 steps),[13a] (−)-sclareolide (80% over 3 steps)[13b] or farnesol (kinetic resolution after electrophilic cyclization, 8% over two steps).[13c]

(−)-Sclareol, the starting substrate of our choice, was isolated on a multigram scale from extracts of *Salvia sclarea*. Furthermore, aiming to ensure the availability of this starting material, we cultivated *Salvia sclarea* for the sustainable production of (−)-sclareol.[14] Thus, following a procedure described by Barrero *et al.*,[13a] we were able to obtain multigram quantities of diol **6**. Key in our approach, we proceeded to study the preparation of the trihydroxy intermediate **7a** through C–H oxidation of the bicyclic diol **6**. Although it was reported that some microorganisms perform 3β-hydroxylations in similar terpenoid systems,[15] the change in the decoration of our starting molecule could drastically alter the specificity of the enzyme–substrate system. Consequently, the desired functionalization was tested with different microorganisms.[16] Our results of incubation of **6** with a growing culture of *Mucor plumbeus* ATCC 4740 are shown in Scheme 3.



Scheme 3 *Mucor plumbeus* C–H oxidation.

Entry	Time	7a	7b
1	10 h	32.7%	
2	14 h	35.4%	
3	16 h	39.5%	
4	**24 h**	**52.0%**	**2.4%**
5	48 h	35.0%	21.0%
6	60 h	18.6%	12.3%
7	5 d	14.8%	21.3%

As can be observed, the proportions of the desired 3β-hydroxy derivative **7a** (established by COSY, HSQC, HMBC, HR-MS) increase with the incubation period up to 24 h. At this point, the overoxidation of the secondary alcohol to ketone becomes a competitive process (entries 4–7). Thus, the best conditions for producing the desired alcohol **7a** were obtained after 24 h of biotransformation (entry 4), when the yield reached 52%. It should be also noticed that the global **7a** + **7b** yield increased up to 54% after 24 h, mainly when it was proven that **7b** was stereospecifically reduced to **7a** with $NaBH_4$ in 83% yield, which also turns **7b** into a valuable synthetic intermediate. The equatorial position of the secondary alcohol function in compound **7a** was evidenced by values of the coupling constants of the H3 peak (J = 10.5, 5.9 Hz).

The next step in our strategy involved the selective protection of the primary alcohol in triol **7a**. After screening a variety of protecting groups, we found that this was not a trivial protection, most likely due to the encumbered environment at C8 and C10. Finally, the desired selectivity was achieved by using pivaloyl chloride[17] in a 1 : 1 mixture of pyridine–CH_2Cl_2 to afford **8** in 76% yield (Scheme 4). Silylation at the C3 hydroxyl and MEM ether protection of the tertiary hydroxyl afforded **9** in an overall yield of 90%. At this point, reductive deprotection of the pivaloyl group afforded the primary alcohol **10**, providing the opportunity to extend the chain at C11.

Swern oxidation of the primary alcohol **10** and Wittig olefination with methyltriphenylphosphonium bromide of the corresponding labile aldehyde[18] allowed us to achieve the required one-carbon homologation. Compound **11** was thus obtained in 85% yield over two steps. The key compound **11** was then coupled *via* palladium catalyzed *B*-alkyl Suzuki–Miyaura[19] with the polyenic vinyl halide **12**[20] in 90% isolated yield to give **13**, a strategy previously reported by us[5c] in the synthesis of myrrhanol A (**2**) and analogues. Moreover, on the basis of the herein described approach, a second generation

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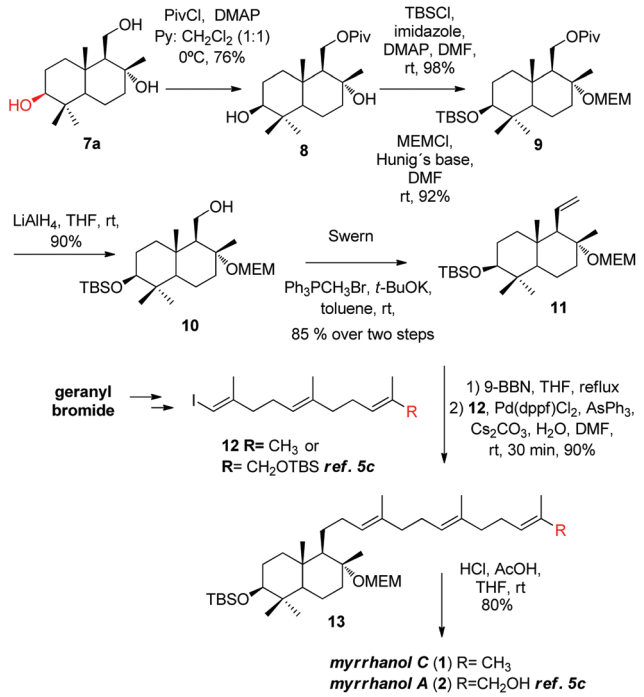

Scheme 4 Synthetic sequence for myrrhanol C and analogues.

synthesis of the potent anti-inflammatory (+)-myrrhanol A (**2**), a triterpene with fewer side effects than hydrocortisone, could be easily conceived by simply changing the linear coupling partner.[21] Mild acid hydrolysis of the protecting groups present in **13** afforded, to our delight, the anti-cancer compound myrrhanol C (**1**) in 80% yield in a concise way. MS, ^{1}H and ^{13}C NMR of our synthetic (+)-myrrhanol C (**1**) coincide completely with those of the natural product.

Conclusions

In summary, we have elaborated on the anti-prostate cancer (+)-myrrhanol C (**1**), a natural product of high bioactive value. The route presented here is scalable and starts from renewable, cheap and commercially available materials. The longest sequence consists of 9 steps with 19% overall yield. Key steps in our convergent synthesis have been the use of a *B*-alkyl Suzuki–Miyaura coupling and a microbial late-stage C–H oxidation, an application of efficient, convenient, selective, and environmentally benign P450 catalysis.

Acknowledgements

This research was supported by the Spanish Ministry of Science and Technology, Project CTQ2010-16818 (BQU). V.D. thanks MICINN for a predoctoral grant. We thank Prof. P. Robert Azerad (René Descartes University, France) for providing *Mucor plumbeus* 4740 ATCC strains. Thanks are also given to M. Jesús González and Prof. Enrique J. Oltra for their collaboration at the early stage of this work.

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20 For experimental details see ESI.†

21 In fact, the synthesis of myrrhanol A using the terminal olefin **11** and the hydroxylated derivative of **12** was reported by us in ref. 5*c*.

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