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Juniperanol: First total synthesis and evaluation in Type 2 Diabetes disease

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ABSTRACT

The first total synthesis of juniperanol, the tricyclic sesquiterpenoid enantiomer of α-cedrol is described. The synthesis relies on stereoselective gold-catalyzed Ohloff-type propargylic ester rearrangement performed on a 10 g scale, and a carbocationic cascade in the presence of acetyl methanesulfonate. The ability of juniperanol to interfere in glucose processes in different cell types is described.

The tricyclic sesquiterpenoids α-cedrene **1** and α-cedrol **2**, isolated from cedar wood *Juniperus rigida* have represented a class of natural products of increasing importance (Scheme 1, top) [1]. Their challenging access and properties [2], even in racemic form for most of the studies, have inspired several international synthetic groups to propose original strategies to construct [5.3.1.0] tricyclic cores, including biomimetic cationic carbocyclization, Diels-Alder, and Pauson-Khand processes, as well as radical cyclization and dearomatization reactions [3]. Juniperanol **3**, the enantiomer of α-cedrol, was isolated and fully identified in 2009 by T.-S. Park and H.-W. Kim from the xylem of evergreen tall conifer *Juniperus chinensis* [4]. The extract of this tree have been used for the treatment of common cold, urinary tract infection, urticaria, dysentery and diarrhea [5]. The Park and Kim group described that juniperanol and compositions thereof prevents and treats hyperlipidemia, fatty liver, diabetes and obesity in different animal models [4]. Considering our interest in discovering new treatments for Type 2 Diabetes Mellitus [6], and our recent work on glucokinase activators [7], we were interested in assessing the glycaemic effects of juniperanol in different cell types. We therefore, embarked in a total synthesis of the natural product. As part of our continued interest in the synthesis of polycyclic derivatives based on atom-economical methodologies [8] and expertise in gold-catalyzed biomimetic carbocationic synthesis [9], we hypothesized that juniperanol **3** could be prepared starting from **4**, *via* a carbocationic cascade [10]. The synthesis of bicyclic derivative **4** was envisioned according to the Fürstner's group highly efficient and elegant strategy [3i], starting from **5**, which could come from (*S*)-citronellal **6** (Scheme 1, bottom). We wish therefore to report herein the first total synthesis of juniperanol and some preliminary biological activities.

First, the readily available citronellal **(*S*)-6** was cleanly and efficiently transformed to allylic bromide **(S)-7** via Fürstner's procedures applied on a very large scale (65 g of chiral **6**, Scheme 2). The Luche reduction step proceeded on a 32 g scale, with reasonable yield. Due to potential industrial applications, we then performed the alkynylation under racemic conditions in the presence of zinc and 3-(trimethylsilyl) propiolaldehyde **8,** leading to the expected two diastereoisomers **5**. We found that the chemical sequence bromination/allylation could be realized on a maximum 15 g scale, which led to a global yield of 45%, analogous to the one obtained on small scale. The alkyne deprotection followed by the esterification reaction could be scaled up to 20 g and afforded the desired isomers **5** in 86% isolated yield. The isomer **(*S*,*R*)-5** and **(*S*,*S*)-5** were not separated at this stage, as we wished to perform only one large scale separation and anticipated an easier separation after the formation of the bicyclic cyclopropane **9**. We optimized the gold-catalyzed Ohloff-type propargylic ester rearrangement [11] by decreasing significantly the amount of gold catalyst [12]. Since the amount of trichloro(pyridine)gold complex could not be reduced down to 5 mol% as a lower conversion of 65% was observed despite a prolonged reaction time (48 h), Scheme 2, we decided to use another source of Au(III) catalyst. We were pleased to find that the stable dichloro(2-pyridinecarboxylato)gold [13] efficiently catalyzed the transformation in a shorter reaction time and delivered the desired bicyclic adducts in 85–88% yield with 2 to 2.5 mol% catalyst. This gold-catalyzed step could thus be performed on a 10 g scale in the presence of 3 mol% Au(III), leading to a 1:1 mixture of stereoisomers **9** in 98% yield. The stereoisomers were separated smoothly at this stage by

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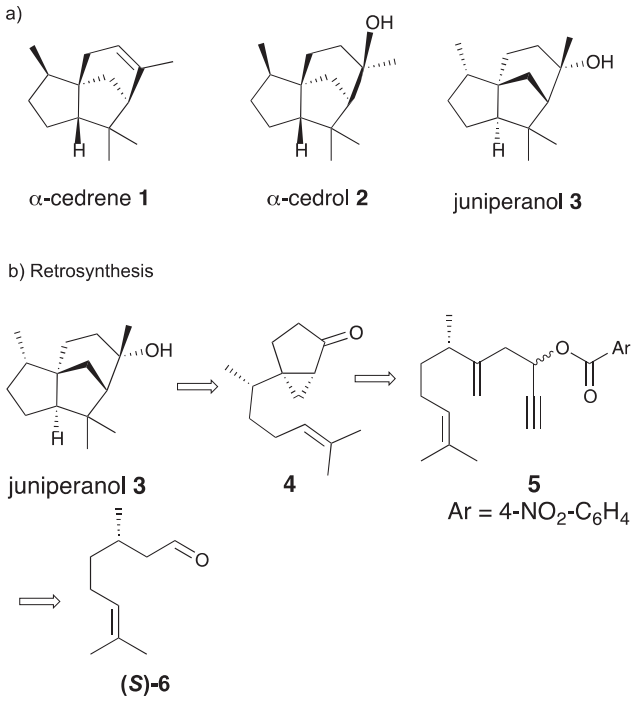
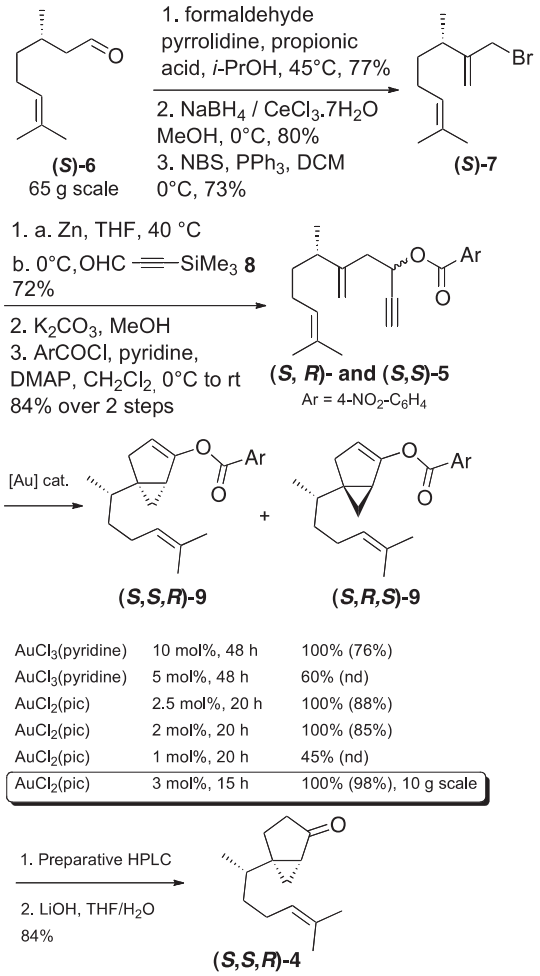


Scheme 1. Example of naturally occurring sesquiterpenes (a). Retrosynthesis of juniperanol 3 (b).

preparative HPLC (see Supporting Information), the desired diastereoisomer **(*S*,*R*,*R*)-9** being isolated on a 18 g scale. The saponification of **(*S*,*R*,*R*)-9** afforded the bicyclic adduct **4**, the precursor of juniperanol skeleton, in 84% isolated yield.

The next step was the challenging carbocationic polycyclization reaction, described by E.J. Corey *et al* in 1973 for the synthesis of racemic cedrol **2** [3b]. However, after several attempts under the described reaction conditions, we could not isolate the desired adduct and therefore revisited this particularly interesting rearrangement (Table 1). The optimization process was performed on a diastereoisomeric mixture of **4/4′** without preparative HPLC separation. Considering the carbocationic-type gold-catalyzed rearrangements [9,11], we evaluated the efficiency of several gold complexes (entries 1–5). Among the various oxophilic Au(III) complexes, in various stoichiometry, full conversions were observed in the case of gold trichloride and chloroauric acid (entries 3–4).

Under a number of reaction conditions, the cyclization of **4/4′** derivatives (Table 1) led to a complex mixture, where barely any trace of juniperanone could be detected. The use of several Brønsted acids such as triflic acid, TFA, methanesulfonic acid and *para*-toluenesulfonic acid, known as efficient additives for Nazarov-type reactions of cyclopropanes [14], in a polar solvent such as acetonitrile, did not give any of the desired products (entries 6–10). The highly complex reaction mixtures that we obtained prompted us to independently prepare cedrone according to Santelli and Tenaglia's procedure [15] and to optimize GC/MS analyses (see Supporting Information), to facilitate the determination of the present of the desired product in the crude reaction mixtures. We reconsidered the use of Lewis acids, including TMSOTf and $AcOSO_2Me$ [16], the acetylating agent described by Corey [3b]. Whereas TMSOTf promoted full consumption of starting material and mostly degradation at room temperature (entry 11), the acetyl methanesulfonate was found to be a milder reagent. Indeed, full conversion was observed in the presence of 5 and 10 equivalents of $AcOSO_2Me$ at



Scheme 2. Large scale synthesis of juniperanol **3** precursor.

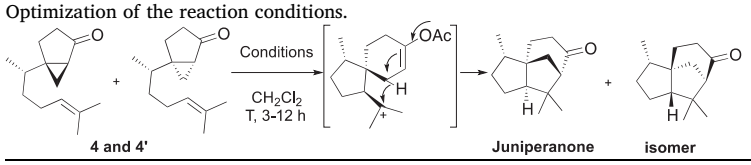
−40 °C and −15 °C (entries 13–15). No or low activities were observed for AcCl (precursor of $AcOSO_2Me$) and Ac_2O (by-product obtained in the synthesis of $AcOSO_2Me$) (entries 16–17). We thoroughly optimized the reaction conditions (temperature, equivalent and concentration) in the presence of the crucial reactant $AcOSO_2Me$ (entries 18–25), and found that the amount of $AcOSO_2Me$ had to be between 7.5 and 10 equivalents with an optimum concentration of 0.02 M. The desired juniperanone could be isolated with an optimized 25% isolated yield (entry 25).

With the optimized reaction conditions established, the carbocationic cyclization was applied to the chiral bicyclic ketone **(*S*,*S*,*R*)-4,** leading to juniperanone adduct **10** in 33% isolated yield (Scheme 3). The last step of methylation of ketone moiety was adapted from Stork *et al* as reported on the synthesis of (+)-cedrol [3c] and nicely afforded the desired juniperanol **3** in 55% yield. Full analyses of juniperanol were compared to commercially available (+)-cedrol to confirm its structure (see Experimental Section).

With a synthetic sample juniperanol in hand, we first checked that it does not affect cell viability looking at lactate dehydrogenase (LDH) levels up to 100 µM in HepG2 cells (Fig. 1a). Second we evaluated potential functional effect on adipocytes and hepatocytes metabolism.

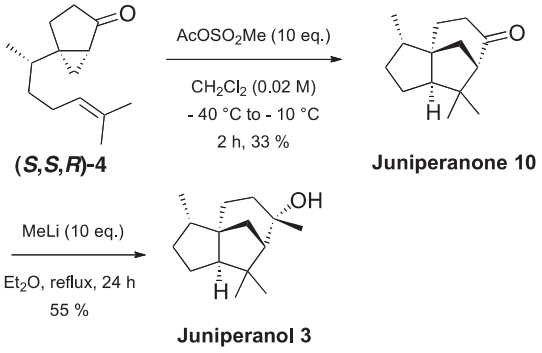
Concentrations of 10 µM juniperanol led to a 60% increase in glucose uptake in 3T3L1 mature adipocytes (Fig. 1b), which is in the same range as that observed in presence of 100 nM insulin. However, it

Table 1
Optimization of the reaction conditions.



Entry	Conditions	T (° C)	Conv. (yld)[a] (%)
1	$AuCl_2$(pic) (10 mol%)	rt	0
2	$AuCl_2$(pic) (10 mol%)	40	0
3	$AuCl_3$ (10 mol%)	rt	100[b]
4	$HAuCl_4$ (10 mol%)	rt	100[b]
5	$AuCl_3$(pyridine) (10 mol%)	rt	0
6[c]	TfOH (1 eq.)	rt	100[b]
7[c]	TfOH (1 eq.)	−40	partial[b]
8[c]	TFA (excess)	rt	0
9[c]	$MeSO_3H$ (1 eq.)	rt	partial[b]
10[c]	$TsOH{\cdot}H_2O$ (1 eq.)	rt	0
11	TMSOTf (5 eq.)	rt	100
12	TMSOTf (5 eq.)	−40	partial[b]
13	$AcOSO_2Me$ (10 eq.)	−40	100
14	$AcOSO_2Me$ (5 eq.)	−40 to rt	100
15	$AcOSO_2Me$ (5 eq.)	−15	100
16	Ac_2O (5 eq.)	rt	0
17	AcCl (5 eq.)	rt	partial[b]
18[d]	$AcOSO_2Me$ (5 eq.)	−40	90 (15)
19[d]	$AcOSO_2Me$ (1.5 eq.)	−40	< 20
20[d]	$AcOSO_2Me$ (3 eq.)	−40	50
21[d]	$AcOSO_2Me$ (7.5 eq.)	−40	100
22[d]	$AcOSO_2Me$ (10 eq.)	−40	100 (15)
23[e]	$AcOSO_2Me$ (5 eq.)	−40	< 20
24[c]	$AcOSO_2Me$ (10 eq.)	−40	50
25[d,f]	$AcOSO_2Me$ (10 eq.)	−40 to −15	100 (25)

[a] Isolated yield.
[b] Complex mixture.
[c] CH_3CN.
[d] 0.02 M.
[e] 0.01 M.
[f] 2 h.



Scheme 3. Final steps for the synthesis of juniperanol **3**.

induces a slight decrease in protein content suggesting a mild cytotoxic effect in this cell type. At 30 μM juniperanol was found to induce a 43% decrease of cAMP-stimulated glucose output in mouse primary hepatocytes. This result is to be compared to Metformin and Insulin that are able to decrease cAMP- stimulated glucose output by 65% at 250 μM and 43% at 100 nM respectively.

In conclusion, we have developed the first total synthesis of juniperanol, a natural sesquiterpene isolated from conifers. The strategy is based on a key gold-catalyzed Ohloff-type propargylic ester rearrangement, which could be performed with up to to 10 g of starting material. The second key step involves a biomimetic carbocationic cascade, optimized in the presence of an electrophilic activator such as acetyl methanesulfonate. The preliminary biological results showed that Juniperanol is able to decrease glucose output in mouse liver and increase glucose uptake in mouse adipocytes which could have a beneficial outcome in metabolic disease.

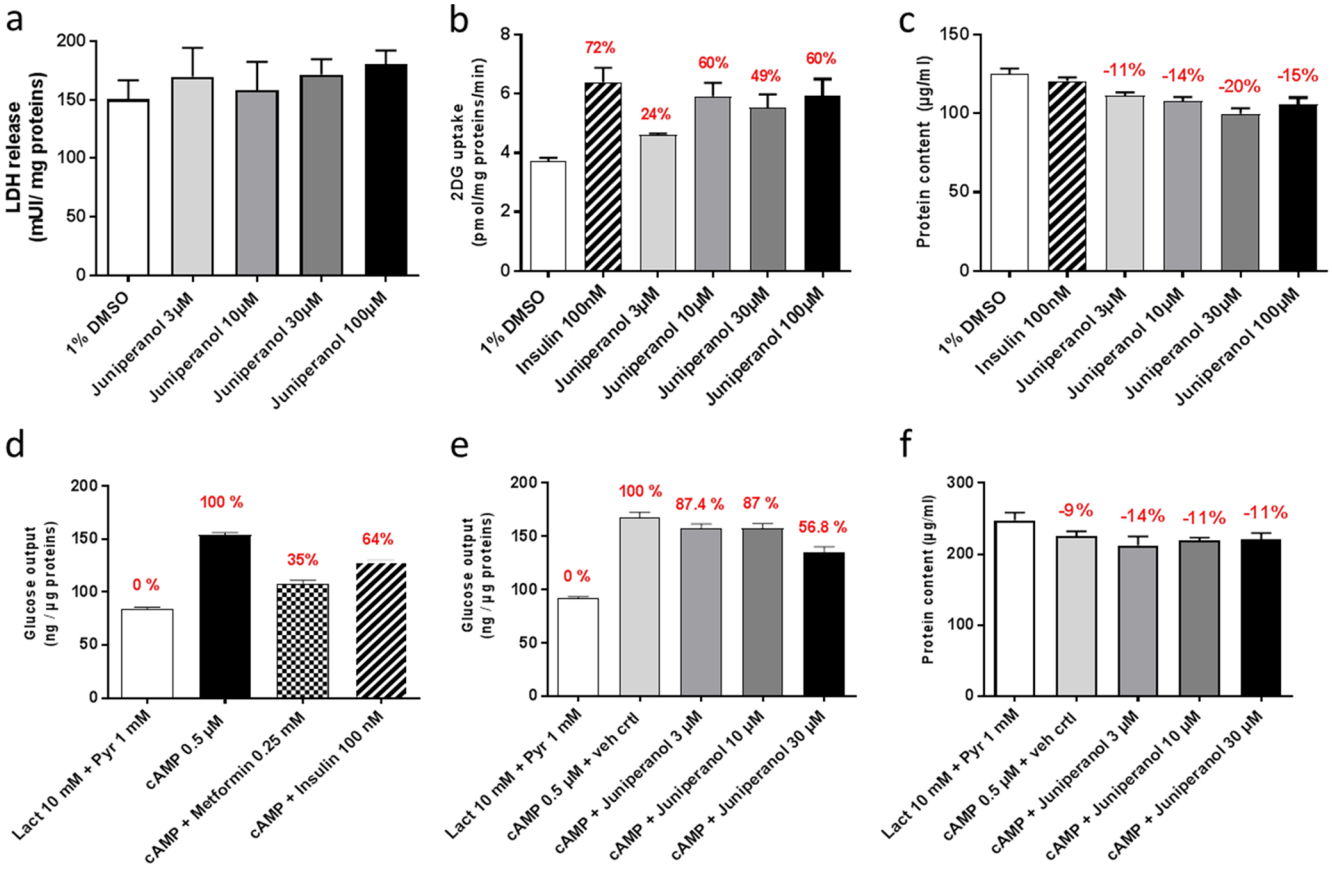


Fig. 1. Assessment of Juniperanol efficacy on metabolic functional assays (a) Cell viability in HepG2 cells in presence of 1% DMSO vehicle control, 3, 10, 30 and 100 µM Juniperanol for 24 h, (b) 3T3L1 adipocyte glucose uptake in presence of 1% DMSO vehicle control, 3, 10, 30 and 100 µM Juniperanol for 24 h compared to 30 min insulin stimulation, (c) Protein content analysis after glucose uptake, (d) cAMP-stimulated glucose output in mouse primary hepatocytes using metformin 0.25 mM and insulin 100 nM as positive controls, (e) cAMP-stimulated glucose output in mouse primary hepatocytes with 0,3% DMSO vehicle control, 3, 10 and 30 µM Juniperanol for 16 h, (f) Protein content analysis after glucose output assay. Results are expressed as mean + /-SEM (triplicates or quadruplicates) n = 1.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103243.

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