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One-step enantioselective synthesis of (4*S*)-isosclerone through biotranformation of juglone by an endophytic fungus

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ABSTRACT

We describe here a direct access to (4*S*)-isosclerone (+)-**1**, an important structural component of several natural products featuring a spirobisnaphthalene ring system. Starting with the commercially available 5-hydroxy-1,4-naphthalenedione (juglone), biotransformation by the isosclerone-producing endophytic fungus *Paraconiothyrium variabile* is described. The absolute configuration of (+)-**1** was determined unambiguously using circular dichroism and by measurement of the optical rotation. Moreover, the biotransformations of other naphthalene derivatives were undertaken and led to the corresponding (4*S*)-hydroxy-1-tetralone. At last, this work brings some insights on the biosynthesis of natural tetralones.

In the course of a program focusing on the structure determination and ecological studies of metabolites from the endophytic fungus *Paraconiothyrium variabile* (1E2a)[1] isolated from the plum yew *Cephalotaxus harringtonia* var. *drupacea* (Siebold & Zucc.) Koidz, we isolated (+)-(4*S*)-isosclerone (**1**), a dihydronaphthalenone commonly found in both enantiomeric forms in plants and fungi. Its absolute configuration was determined by comparison of the circular dichroism (CD) spectra with those available from the literature.[2] Indeed, isosclerone was first isolated from *Sclerotina sclerotinium* as a new bioactive metabolite with plant growth regulating properties[3] and later in a large variety of plants and fungi.[4–6] Recently, the enantiomer (−)-(4*R*)-regiolone **2** was also reported as a phytotoxin of *Botrytis cinerea*[7] and configurational assignment of its stereocenter was unambiguously provided by ab initio computational prediction of its theoretical optical rotation and electronic CD spectra (Fig. 1).[8]

Interestingly, the phytotoxic activity was correlated to the absolute configuration of each isomer.[2]

These compounds are also of particular interest as building blocks for more complex natural compounds featuring a spirobisnaphthalene structure such as preussomerins, palmarumycins, or diepoxins which have significant biological activities.[9] Nevertheless compound **1** is weakly produced in fungal cultures (from 0.4 to 2 mg L^{-1}).[3,8] Moreover a racemic synthesis of compounds **1** and **2** was achieved in four steps from 2-(acetoxy)-6-(bromomethyl)benzoate and 8.8% overall yield[10] or in one step from the chemical reduction of juglone (40% overall yield),[4] but no stereoselective synthesis of (4*S*)-isosclerone (**1**) has been reported to date.

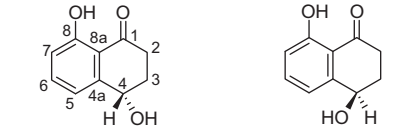

Figure 1. Structure of isosclerone (**1**) and regiolone (**2**).

Microbial transformations of aromatic hydrocarbons like naphthalene have been the subject of extensive research.[11] Endophytic fungi are able to act as biocatalysts and can chemically modify compounds.[12] For instance, biotranformations of monoterpenoids,[13,14] alkaloids,[15] and taxoids,[16] by endophytic fungi were reported.[17] More relevant to this work is the obtention of the racemic 4-hydroxy-1-tetralone by the biotransformation of naphthalene using *Streptomyces griseus* NRRL 8090.[11]

We wish to report herein the first one-step enantioselective synthesis of compound **1**, which can be viewed as an ideal synthesis, through direct biotransformation of juglone (**3**) by the endophytic fungus *Paraconiothyrium variabile*. Moreover, biotransformations of analogues were also undertaken.

A kinetic study of the biotransformation of juglone by *P. variabile* was first performed. The biomass was obtained by culturing

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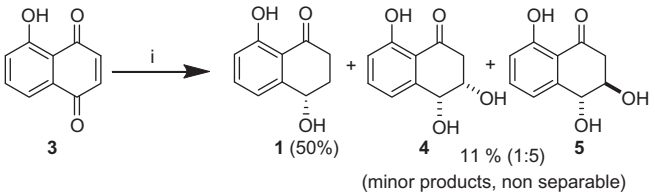


Scheme 1. Biotransformation of juglone (**3**). (i) *P. variabile* resting cells, 48 h, phosphate buffer, 25 °C.

the fungus for 4 days in YMS culture medium and incubation was conducted in resting cells in phosphate buffer.[18] Juglone (**3**) (17 mg) was then added and biotransformation reactions were monitored by HPLC-MS. At 48 h, juglone was totally consumed and the chromatogram revealed the presence of two distinct peaks.

Purification on silica gel (CH_2Cl_2:MeOH 95:5) led to compound **1** (8.6 mg, 50% yield) with high enantioselectivity (>98% ee)[19] and to the mixture of two constitutional isomers **4** and **5** (2 mg, 11%) nonseparable in our hands (Scheme 1). Structure of the isolated compound **1** was established on the basis of ^1H and ^{13}C NMR which confirmed its structure as either isosclerone already isolated from *P. variabile*[20] or regiolone. The absolute configuration of **1** was assigned by circular dichroism and comparison to a pure sample of natural isosclerone. As depicted in Figure 2, the CD spectra were superimposable and showed characteristic strong positive Cotton effect at 213 nm ($\Delta\varepsilon = +15.90$) and a strong negative band at 258 nm ($\Delta\varepsilon = -3.40$) suggesting that compound (**1**) is (+)-(4*S*)-isosclerone. Moreover, the configuration of C-4 deduced by the CD spectrum is in good agreement with the value found experimentally of optical rotation in $CHCl_3$ $[\alpha]_D^{22}$ +18.5 (*c*, 3.25) (lit. $[\alpha]_D^{27}$ +24.5).[8]

Concerning the mixture of the two diasteroisomers **4** and **5**, HR-ESI-MS data pointed out a single protonated ion $[M+H]^+$ at *m*/*z* 195.0649 corresponding to the molecular formula $C_{10}H_{11}O_4$ (calcd 195.0658 for $C_{10}H_{11}O_4$). This mixture was also analyzed by ^1H NMR and revealed the presence of two constitutional isomers with a ratio 1:5 (**4:5**). The ^{13}C NMR spectrum exhibited for each compound 10 carbon resonances including one carboxyl, two oxymethines and three quaternary. The ^1H–^1H COSY spectrum was indicative of two spin systems as represented in Figure 3. These substructures were assembled from the HMBC data and led to the characterization of 3,4,8-trihydroxy-1-tetralone **4** and **5**.

The relative configuration of the diol moiety of **4** and **5** was determined by the analysis of NOE correlations and coupling constant values. Indeed for **4**, a NOE correlation between the two oxymethine protons was observed associated to a low coupling constant (J = 3.1 Hz) suggesting a *syn* relationship for the diol. On the other hand, for the oxymethine protons of **5** a coupling constant of 8.1 Hz associated to the absence of NOE correlation implied a trans relative configuration. Interestingly, these two compounds have already been isolated as natural products from fungi.[21,22]

Biosynthetically, this experiment is interesting if we consider the fact that *P. variabile* is a natural producer of (+)-isosclerone as previously shown by us.[20] Indeed juglone may be an intermediate in aromatic pentaketide biosynthesis in fungi even though we did not isolate it during our previous work from *P. variabile*. Of course prior to the biotransformation experiment, we checked and confirmed that the used resting cells did not contain any constitutive isosclerone residue.

Eventually, the biotransformation was successfully conducted at semi-preparative scale and led to relevant quantity of isosclerone.[23]

The bioconversion pathway is interesting as juglone (**3**) has to undergo two biocatalytic reductions through two possible pathways. The first one would involve the reduction of carbon–carbon double bond followed in the second step by an infrequent reduction of polyhydroxynaththalene which has been only reported in melanin biosynthesis.[24] In contrast, the second pathway, would involve the reduction of the carbonyl at C-4 followed by the one of the double bond. Compounds **4** and **5** may thus arise from nonstereoselective spontaneous hydration of the putative enone intermediate, with compound **5** being thermodynamically favored.

Moreover, with the aim to better characterize the scope of the biotransformation by this endophytic fungus and the biotransformation pathway, other naphthalene analogues were tentatively transformed. In this context, 1,4-naphtoquinone (**6**) was added to the cell suspension of *P. variabile* and (+)-(4*S*)-hydroxy-1-tetralone (**7**) was promptly isolated in a good yield (42 mg, 48% yield) with 60% enantiomeric excess (Scheme 2).[25] This result shows that the reduction of a symmetric prochiral diketone is less stereoselective than the hydroxylated one.

Addition of 1,4-dihydroxynaphtalene (**8**) to *P. variabile* also led to compound (**7**). This was likely due to the conversion of the 1,4-dihydroxynaphtalene (**8**) into the corresponding quinone (**6**). Unfortunately, this conversion was also observed under inert atmosphere and did not permit to discriminate between the both the proposed pathways above. Moreover a trial with tetralone (**9**) did not lead to any product.

In conclusion, we have reported here an original and efficient use of a biotransformation by an endophytic fungus for the enantioselective synthesis of (+)-isosclerone (**1**) from juglone (**3**). Additional experiments led to the biotransformation of the 1,4-naphthoquinone (**6**) into 4-hydroxy-1-tetralone (**7**) also with good

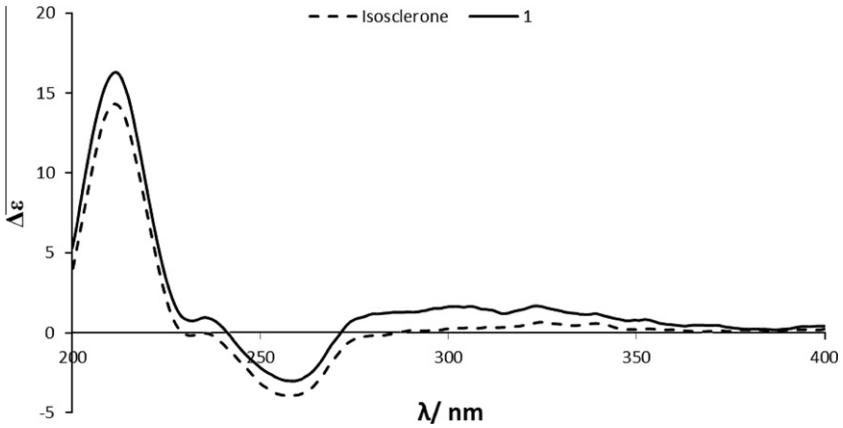


Figure 2. CD spectra of (**1**) (solid line) and pure sample isosclerone (hatched line) recorded in MeOH at 22 °C (*c*, 10^{-2}).

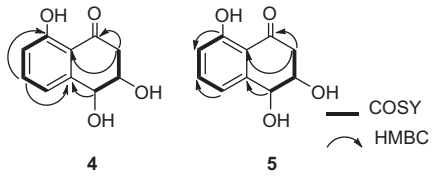
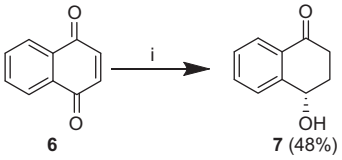


Figure 3. Key ^{1}H–^{1}H COSY (bold bonds) and HMBC correlations (H → C) for **4** and **5**.



Scheme 2. Biotransformation of 1,4-naphtoquinone (**6**). (i) *P. variabile* resting cells, 8 h, phosphate buffer, 25 °C.

yields. It should be noted that these compounds could be the structural components of several natural products featuring a spirobisnaphthalene skeleton.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.12.038.

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23. Two hundred milliliters of YMS-culture medium in a 500 mL flask containing (g/L) glucose 16, yeast extract 4, malt extract 10, and soybean peptones 5 were inoculated with 200 μL glycerol suspension of *P. variabile* and incubated at 27°C and 160 rpm in an orbital shaker. After 96 h biomass was collected by filtration and cells (90 g) were then suspended in phosphate buffer (0.2 M, pH 6.5, 100 mL) and juglone (100 mg) dissolved in DMF (100 μL) was added. Biotransformations were performed at 27°C in an orbital shaker. After 48 h of incubation, the cell suspension was filtered. Thus, the filtrate was saturated with NaCl, extracted with ethyl acetate (three times) and the organic phases were combined, dried over Na_2SO_4. After evaporation, the product was purified by flash chromatography (CH_2Cl_2/MeOH 9:1) (44.2 mg, 44%). $[\alpha]_D^{20}$ +18.5 (*c*, 3.25, $CHCl_3$).
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25. Biomass (90 g) obtained as described above was suspended in phosphate buffer (0.2 M, pH 6.5, 90 mL). 1,4 Naphtoquinone (90 mg) dissolved in DMF (500 μL) was also added in buffer (90 mL) and this solution was poured to the cell suspension. Biotransformations were then performed at 27°C in an orbital shaker, stopped after 8 h and treated as above. After evaporation, the product was purified by flash chromatography, elution with CH_2Cl_2/MeOH 97:3 (42 mg, 48%), $[\alpha]_D^{20}$ +21.3 (*c*, 0.47, $CHCl_3$) (Lit. $[\alpha]_D^{20}$ +23.5, *c*, 0.7, $CHCl_3$).[26] Enantiomeric excess determined by GC analysis. Colonm CP-chirasil-Dex CB 25 m 0.32 mm, 25 μm, carrier gas He 15psi, oven temperature 140°C (20 min), gradient 3°C/min 150 °C. Retention times (*S*)-isomer 26.2 min, (*R*)-isomer 27.3 min.
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