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PAPER

Biotransformation of clovane derivatives. Whole cell fungi mediated domino synthesis of rumphellclovane A†

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Here we describe the biotransformation of clovane derivatives by filamentary fungi *Pestalotiopsis palustris* and *Penicillium minioluteum*, and the application of the latter to the synthesis and determination of the absolute configuration of rumphellclovane A (**2**). Methoxyclovanol (**1**), a growth inhibitor of the phytopathogen *Botrytis cinerea*, is metabolised by *P. palustris* to yield rumphellclovane A (**2**), a natural compound recently isolated from the gorgonian coral *Rumphella antipathies*, two new compounds, (1*R*,2*S*,5*S*,8*R*,9*S*,10*R*)-2-methoxyclovane-9,10-diol (**5**) and (1*S*,2*S*,5*S*,7*R*,8*R*,9*R*)-2-methoxyclovane-7,9-diol (**6**), hydroxylated in positions not easily accessed by classic synthetic chemistry, and clovanodiols **3** and **4**. *P. minioluteum* is able to selectively transform methoxyclovanol (**1**) into clovanodiols **3** and **4** and, in turn, lactone **8**, the putative intermediate in the above mentioned synthesis of rumphellclovane A (**2**), into compound **2** *via* a domino process. The ability of *P. minioluteum* to carry out the cleavage of ethers on clovane derivatives is also evaluated.

Introduction

Clovane derivatives are a group of terpenes which have displayed interesting biological activities.[1] Compounds with a clovane skeleton have exhibited activity as growth inhibitors of the fungus *Botrytis cinerea*, a serious plant pathogen that has developed resistance to some commercial fungicides.[2] The biotransformation and detoxification of some bioactive clovanes by *B. cinerea* has already been studied.[3] These studies have revealed that the cleavage of ether function at C-2 of the clovane skeleton and redox reactions at C-9, are key detoxification reactions carried out by the fungus. These results suggest that active clovane derivatives can be considered as a new class of non persistent fungistatic compounds.[1a,3]

An approach to the development of this class of fungistatic agents against *B. cinerea* requires the evaluation of a number of clovane skeleton derivatives featuring different oxidation patterns to those shown on clovanes metabolised by *B. cinerea*,[4] irrespective of cleaving or not cleaving an ether function at the C-2 position. Here, the use of other microorganisms as biotransformation agents can be a useful methodology insofar as these can give rise to products oxidised at positions not easily accessed by classic synthetic chemistry, with the added advantage of mild reaction conditions.[5]

The cleavage of a C–O bond of the ether functional group is a well known reaction in organic synthesis and many methods have been developed.[6] However, these methods entail disadvantages such as the utilization of harsh conditions, use of toxic, expensive, and unstable reagents or catalysts and the formation of mixtures and low product yield. Microbiological methods, potentially milder and more economical, have typically been applied to the breakdown of agro-chemicals and raw materials like lignin,[7] and only recently there are reports of preparative applications for the cleavage of ether groups,[8] even selectively.[9]

The microorganisms responsible for ether cleavage can potentially be useful as tools in synthetic methodology such as the transformation of methoxy into hydroxy functional groups.

This paper describes the biotransformation of (1*S*,2*S*,5*S*,8*R*,9*R*)-2-methoxyclovan-9-ol (**1**) by the fungi *Pestalotiopsis palustris* and *Penicillium minioluteum*, evaluates the ability of the latter to carry out the cleavage of ethers on clovane derivatives and describes its application to the synthesis and determination of the absolute configuration of rumphellclovane A (**2**), a novel clovane-related derivative isolated from the gorgonian coral *Rumphella antipathies*.[10]

Results and discussion

(1*S*,2*S*,5*S*,8*R*,9*R*)-2-Methoxyclovan-9-ol (**1**), prepared from (–)-caryophyllene oxide,[11] was used as the substrate for

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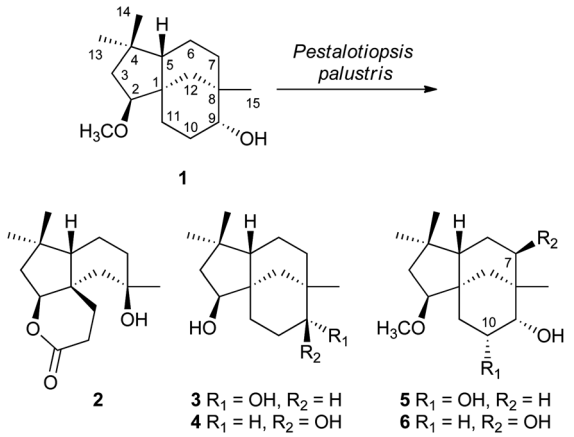
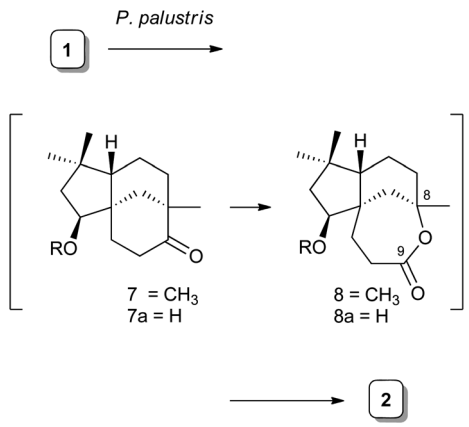


Scheme 1



Scheme 2

biotransformations with *P. palustris* and *P. minioluteum*. The incubation of **1** by *P. palustris* under surface culture conditions gave rise to compounds **2** (2%), **3** (3%), **4** (10%), **5** (1%) and **6** (4%)[5] after ten days (Scheme 1). Analysis of the spectroscopic data of compound **2** showed that this compound was identical to rumphellclovane A,[10] a plausible clovane derivative, recently isolated from the gorgonian coral *Rumphella antipathies* and whose common biosynthetic origin was proposed. Rumphellclovane A (**2**) has a rearranged lactone skeleton probably formed as a result of a four-part, domino-process reaction: demethylation of the methoxyl group at C-2, oxidation of the hydroxy group at C-9, Baeyer–Villiger oxidation at the C-8/C-9 bond and further translactonization (Scheme 2).

Compounds **3** and **4**, identified by analysis of their spectroscopic data respectively as (1*S*,2*S*,5*S*,8*R*,9*R*)-clovane-2,9-diol (**3**)[12] and (1*S*,2*S*,5*S*,8*R*,9*S*)-clovane-2,9-diol (**4**),[3c] resulted from the demethylation of the starting material at position C-2 (compound **3**), while further epimerization at C-9 led to compound **4**.

Two additional metabolites, **5** (1.0 mg, 1%) and **6** (4.8 mg, 4.8%), were obtained from the biotransformation of methoxyclovanol (**1**) by *P. palustris*. The ^{13}C NMR spectra of these novel compounds showed 16 carbons, four methyl (one methoxyl), five methylene, four methyne and three quaternary carbons, and their HREIMS gave ion peaks consistent with the formula $C_{16}H_{28}O_3$.

Table 1 ^{1}H and ^{13}C NMR data, (1*R*,2*S*,5*S*,8*R*,9*S*,10*R*)-2-methoxyclovane-9,10-diol (**5**) and (1*S*,2*S*,5*S*,7*R*,8*R*,9*R*)-2-methoxyclovane-7,9-diol (**6**) (*J* values in parentheses, in Hertz)

	(1*R*,2*S*,5*S*,8*R*,9*S*,10*R*)-2-Methoxyclovane-9,10-diol (**5**)[a]		(1*S*,2*S*,5*S*,7*R*,8*R*,9*R*)-2-Methoxyclovane-7,9-diol (**6**)[b]	
Position	δ_H (600 MHz)	δ_C (150 MHz)	δ_H (400 MHz)	δ_C (100 MHz)
1		45.9		46.5
2	*α*: 3.38 dd (5.6, 10.0)	90.9	*α*: 3.19 dd (3.9, 5.4)	89.8
3	*α*: 1.71 dd (5.6, 12.0) *β*: 1.49 dd (10.0, 12.0)	45.2	*α*: 1.79 dd (5.4, 13.7) *β*: 1.59–1.68 m	44.1
4		38.4		39.9
5	*β*: 1.47 m	52.7	*β*: 1.84–1.86 m	50.8
6	*a*: 1.32–1.41 m *b*: 0.85–0.90 m	21.4	*α*: 1.95 ddd (1.6, 7.6, 14.8) *β*: 1.64 m	31.7
7	*α*: 1.37 m *β*: 1.18 m	33.6	*α*: 3.69 dd (7.6, 11.0)	76.5
8		36.1		40.2
9	*β*: 3.21 d (3.4)	78.5	*β*: 3.88 brs	68.5
10	*β*: 3.94 ddd (3.4, 5.4, 12.3)	68.0	*α*: 1.70–1.80 m *β*: 2.02–2.15 m	30.0
11	*α*: 1.54 t (12.3) *β*: 1.35 m	36.7	*a,b*: 1.60–1.65	27.9
12[c]	*a*: 0.96 d (12.7) *b*: 1.59 d (12.7)	36.6	*a*: 0.96 m *b*: 1.54 dd (1.6, 13.3)	38.4
13α	0.87 s	25.7	0.98 s	27.2
14β	1.03 s	31.6	1.01 s	32.7
15	0.98 s	28.9	1.07 s	25.5
–OCH_3	3.33 s	58.5	3.27 s	57.2

[a] CD_3OD. [b] $CDCl_3$. [c] Greek letters are used to indicate relative stereochemistry; where no clear assignment of relative stereochemistry can be made. Latin letters (a, b) are used to distinguish between diastereotopic protons connected to the same carbon (same position).

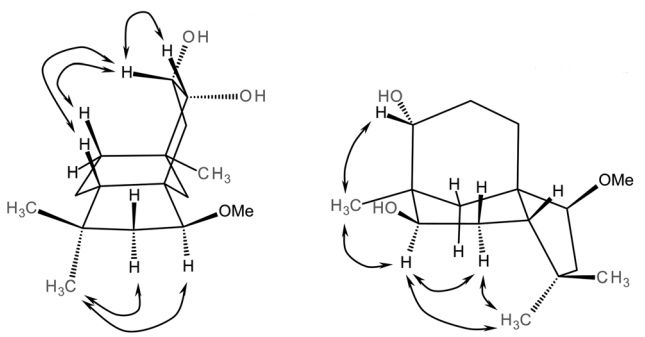


Fig. 1 Selected NOESY correlations found for compounds **5** and **6**.

The structures of compounds **5** and **6** were determined using a combination of 1D and 2D NMR spectroscopy (Table 1, Fig. 1). Comparison of δ_C from incubated compound **1** and compound **5**, pointed to multiplicity as well as chemical changes in resonance for C-10 from δ_C 26.0 (t) in compound **1**[11] to δ_C 68.0 (d) in compound **5**. The presence of a hydroxy group at this position was confirmed by COSY correlations of signal at δ_H 3.94 (H-10) with those at δ_H 1.35 (H-11β), δ_H 1.54 (H-11α) and δ_H 3.21

(H-9β) and an HMBC correlation of C-10 with signals at δ_H 1.35 (H-11β) and δ_H 1.54 (H-11α). NOE effects among signals δ_H 3.94 (H-10) and δ_H 1.18 (H-7β), δ_H 1.47 (H-5β) and δ_H 3.21 (H-9β) supported the assignment of C*H*OH at C-10 as H-10β (Fig. 1) and confirmed the structure of this compound as (1*R*,2*S*,5*S*,8*R*,9*S*,10*R*)-2-methoxyclovane-9,10-diol (**5**).

Comparison of δ_C of incubated compound **1** and compound **6**, pointed to multiplicity and chemical changes in resonance for C-7 from δ_C 33.1 (t) in compound **1**[11] to δ_C 76.5 (d) in compound **6**. The presence of a hydroxy group at this position was confirmed by COSY correlations of the signal at δ_H 3.69 (H-7) with those at δ_H 1.64 (H-6β) and δ_H 1.94 (H-6α) and HMBC correlations of C-7 with signals at δ_H 0.96 (H-12a), δ_H 1.07 (H_3-15), δ_H 1.54 (H-12b), δ_H 1.64 (H-6β), and δ_H 1.94 (H-6α). NOE effects among signals δ_H 3.69 (H-7) and δ_H 0.98 (H_3-13α), δ_H 1.07 (H_3-15) and δ_H 1.95 (H-6α) supported the assignment of C*H*OH at C-7 as H-7α (Fig. 1) and confirmed the structure of this compound as (1*S*,2*S*,5*S*,7*R*,8*R*,9*R*)-2-methoxyclovane-7,9-diol (**6**).

The incubation of compound **1** by *P. minioluteum* under surface culture conditions for ten days led only to compounds **3** and **4**. Interestingly, the transformation was more selective and the yields were higher (**3**, 17% yield and **4**, 11% yield) than those observed for the biotransformation of compound **1** by *P. palustris*.

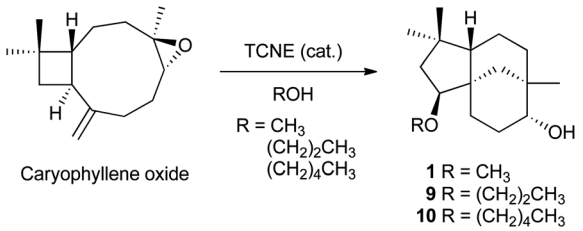
In order to evaluate whether the C–O bond cleavage ability of *P. minioluteum* can be extended to the other side chains other than methyl, we prepared and incubated clovane derivatives **9**, **10** and **11** with whole cell cultures of *P. minioluteum*. Compounds **9** and **10**, not previously described in the literature, were prepared by TCNE catalysed solvolysis of (−)-caryophyllene oxide with n-propanol and n-pentanol, respectively (Scheme 3).[11] Molecular formulas $C_{18}H_{32}O_2$ and $C_{20}H_{36}O_2$ were assigned to compounds **9** and **10** respectively as derived from their HREIMS. Structural elucidation of compounds **9** and **10** was achieved by a combination of 1D and 2D NMR spectroscopy and confirmed the structure of these compounds as (1*S*,2*S*,5*S*,8*R*,9*R*)-2-propoxyclovan-9-ol (**9**), (1*S*,2*S*,5*S*,8*R*, 9*R*)-2-pentoxyclovan-9-ol (**10**). The compound (1*S*,2*S*,5*S*,8*R*, 9*R*)-2-(2′-(*p*-nitrophenoxy)ethoxy)clovan-9-ol (**11**) was prepared as reported in a previous publication.[3a]

In three independent experiments, 60 mg of compounds **9–11** were incubated with *P. minioluteum* in two Roux-type bottles for 10 days (Scheme 4), the extracts were studied by gas chromatography coupled to mass spectrometry, column chromatography and HPLC and further spectroscopic characterization of the compounds isolated was carried out. Biotransformation of propoxyclovanol **9** also produced clovanodiols **3** and **4** but at lower yields than those observed in the biotransformation of compound **1** under comparable conditions (Table 2). In contrast, independent biotransformation of compounds **10** and **11** with *P. minioluteum* did not yield any transformed clovane derivatives, only leading to the recovery of starting material. Even though no obvious solubility problems were observed, their higher lipophilicity could explain the lack of metabolism through mechanisms observed for compounds **1** and **9**. Interestingly, both compounds present calculated[13] log *P* values close to the upper limit of log *P*[14] established by the Lipinsky "rule of five" for oral availability of drugs[15] which provides a guide in the search for active principles (compound **10**, log *P* = 5.004; compound **11**, log *P* = 4.824). On the other hand, log *P* for compounds **1** (log *P* = 3.061) and **9** (log *P* = 3.939), which are metabolised by the fungus, fall well within the margins of the Lipinski rule for log *P*. These results seem to indicate that the length and nature of the side chain of the clovane skeleton at C-2 have a significant influence on the ability of *P. minioluteum* to remove it; only substrates with a small enough side chain as those presented in compounds **1** and **9** seem to be metabolized by the fungi.

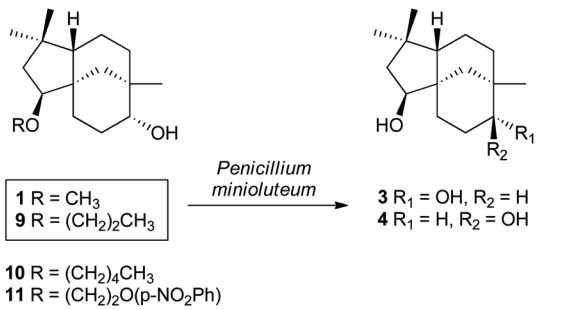
Table 2 Metabolites of clovanes by *P. minioluteum*

Substrates	Metabolites (yield, %)
1	**3** (17), **4** (11)
9[a]	**3** (8), **4** (2)
10	—[b]
11	—[b]

[a] 7 mg of compound **9** were recovered. [b] No metabolites were obtained.

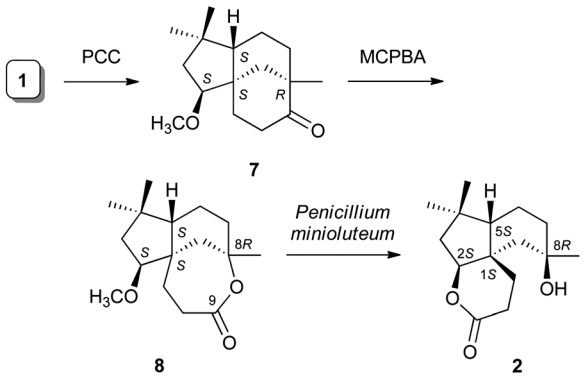


Scheme 3



Scheme 4

Since *P. minioluteum* was capable of interaction at the C-2 and C-9 positions on compounds with clovane skeletons yielding reasonable amounts of biotransformation product, we tested a plausible application of this fungus as a biocatalyst to a biomimetic synthesis of rumphellclovane A (**2**).[10] Compound **1** was oxidized with PCC to give ketone **7**[3c] which, in turn, was transformed by treatment with MCPBA to lactone **8** (67% yield), a light yellow solid with molecular formula $C_{16}H_{26}O_3$ as derived from its HREIMS. IR spectrum of this compound suggested the presence of a lactone group (1711, 1186 cm^{-1}). The shift in C-8 resonance from δ_C 44.4 in compound **7** to δ_C 80.0 in compound **8** in its ^{13}C NMR spectrum indicated that lactonisation was accomplished with insertion of an oxygen atom between C-9 and C-8. Further analysis of 1D and 2D NMR spectroscopy allowed us to confirm the structure of this compound as (1*S*,2*S*,5*S*,8*R*)-8,9-seco-8-hydroxy-2-methoxyclovane-9-lactone (**8**), a derivative of the hypothetical biosynthetic precursor of rumphellclovane A (**2**).[10]



Scheme 5

Compound **8** was then incubated with a surface culture of *P. minioluteum* for 10 days. As a result, a compound with identical spectroscopic and physical data to rumphellclovane A (**2**) was obtained at a yield of 16% *via* a domino demethylation/translactonization process. These results support the hypothetical biosynthetic route proposed for compound **2**.[10] Anyway, either compound **8** or **8a** could be envisaged as intermediates in the transformation of methoxyclovanol (**1**) to rumphellclovane A (**2**) by *P. palustris*.

Additionally, the optical rotation of compound **2**, obtained through microbiological transformation (Schemes 1 and 5), showed the same sign and magnitude to that described for the marine natural product[10] thus supporting the absolute configuration for rumphellclovane A as 1*S*,2*S*,5*S*,8*R*.

Conclusion

(1*S*,2*S*,5*S*,8*R*,9*R*)-2-Methoxyclovan-9-ol (**1**) is metabolised by a whole cell culture of *P. palustris* to yield rumphellclovane A (**2**), a natural compound recently isolated from the gorgonian coral *R. antipathies*, two new compounds, (1*R*,2*S*,5*S*,8*R*,9*S*,10*R*)-2-methoxyclovane-9,10-diol (**5**) and (1*S*,2*S*,5*S*,7*R*,8*R*,9*R*)-2-methoxyclovane-7,9-diol (**6**), hydroxylated at positions not easily accessed by classic synthetic chemistry, and the previously described (1*S*,2*S*,5*S*,8*R*,9*R*)-clovane-2,9-diol (**3**) and (1*S*,2*S*,5*S*,8*R*,9*S*)-clovane-2,9-diol (**4**).

We also evaluated the ability of the fungus *P. minioluteum* to perform the cleavage of an ether function at C-2 on clovane derivatives. Results show that the length and nature of the side chain have a significant influence on the reaction. We also applied the fungus *P. minioluteum* in a biomimetic synthesis of rumphellclovane A (**2**), where the fungus was able to mediate the demethylation/translactonization domino reactions which transformed lactone **8** into the natural product. Finally, this synthesis led to the assignment of the absolute configuration of rumphellclovane A (**2**) as 1*S*,2*S*,5*S*,8*R* and supported its proposed biosynthetic route.

Experimental

General procedures

Melting points were measured with a Reichert-Jung Kofler block and are uncorrected. Optical rotations were determined with a Perkin-Elmer 341 polarimeter. $[\alpha]_D$ Values are given in 10^3 degree cm^2 g^{-1}, concentration of the sample in 10^2 cm^{-3} g. IR spectra were recorded on a Perkin-Elmer Spectrum BX FT-IR spectrophotometer. ^{1}H and ^{13}C NMR measurements were obtained on Varian INOVA 400 and INOVA 600 NMR spectrometers with $SiMe_4$ as the internal reference. *J* values are given in Hz. NMR assignments were made by a combination of 1D and 2D techniques and by comparison with those made for previously described compounds, where appropriate. Mass spectra were recorded on a Finnigan Voyager spectrometer at 70 eV. High-resolution mass spectra were recorded on a Micromass Autospec spectrometer at 70 eV. HPLC was performed with a Hitachi/Merck L-6270 apparatus equipped with a UVVIS detector (L 4250) and a differential refractometer detector (RI-71). TLC was performed on Merck Kiesegel 60 F254, 0.2 mm thick. Silica gel (Merck) was used for column chromatography. Purification by HPLC was accomplished using a Si gel column (LiChrospher Si 60, 10 μm, 1 cm wide, 25 cm long).

Synthesis of the substrates

(1*S*,2*S*,5*S*,8*R*,9*R*)-2-Methoxyclovan-9-ol (**1**) (1*S*,2*S*,5*S*,8*R*)-2-methoxyclovan-9-one (**7**) and (1*S*,2*S*,5*S*,8*R*,9*R*)-2-(2′-(*p*-nitrophenoxy)ethoxy)clovan-9-ol (**11**) were prepared according to procedures described in the literature.[3c,11]

(1*S*,2*S*,5*S*,8*R*)-8,9-Seco-8-hydroxy-2-methoxyclovane-9-lactone (8). 327 mg (1.31 mmol) of compound **7** were dissolved by stirring in 40 mL of CH_2Cl_2 at room temperature. Then, 2645 mg (15.33 mmol) of MCPBA were added and the reaction monitored by TLC. After 25 h the reaction mixture was neutralized with Na_2CO_3, and extracted with ethyl acetate. The organic layer was washed with a saturated solution of $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. Column chromatography of the crude reaction mixture on silica gel, eluted with a gradient mixture of petroleum ether–EtOAc yielded compound **8** (233 mg, 0.87 mmol, 67% yield). Obtained as a light yellow solid $[\alpha]_D^{25}$ −21.4 (*c* 0.1, CH_3OH); IR (film) ν_{max}/cm^{-1} 1711, 1186 (lactone); δ_H (400 MHz; $CDCl_3$,) 0.87 (3H, s, H_3-13α), 1.07 (3H, s, H_3-14β), 1.09 (1H, m, H-6a), 1.39 (3H, s, H_3-15), 1.42 (1H, m, H-11a), 1.45 (1H, t, *J* 11.5, H-3β), 1.59 (1H, m, H-6b), 1.55 (1H, m, H-5β), 1.61 (1H, d, *J* 15.3, H-12a), 1.77 (1H, dd, *J* 5.6 and 11.5, H-3α), 1.79 (1H, m, H-7a), 1.85 (1H, m, H-11b), 1.97 (1H, d, *J* 15.3, H-12b), 2.02 (1H, m, H-7b), 2.68–2.83 (2H, H-10a,b), 3.32 (3H, s, OCH_3), 3.35 (1H, dd, *J* 5.6 and 11.5, H-2α); δ_C (100 MHz, $CDCl_3$) 20.3 (t, C-6), 25.5 (q, C-13), 30.8 (t, C-11), 31.0 (q, C-14), 32.2 (q, C-15), 32.8 (t, C-10), 35.5 (t, C-7), 36.8 (s, C-4), 43.8 (t, C-3), 44.8 (s, C-1), 46.9 (t, C-12), 51.0 (d, C-5), 58.3 (q, –OCH_3), 80.0 (s, C-8), 90.7 (d, C-2), 174.7 (s, C-9); HMBC (selected correlations) C-8 → H-7a, H-7b, H-12a, H-12b, H_3-15; C-9 → H-10a, H-10b, H-11a, H-11b; C-12 → H-2α, H_3-15; EIMS *m*/*z* 266 (M^+, 2%), 251 (M^+ − 15, 3), 248 (M^+ − 18, 2), 210 (8), 164 (44), 161 (55), 119 (65), 105 (100), 99 (96); HREIMS *m*/*z* 266.1981 $[M]^+$ (calcd for $C_{16}H_{26}O_3$, 266.1882).

(1*S*,2*S*,5*S*,8*R*,9*R*)-2-Propoxyclovan-9-ol (9), (1*S*,2*S*,5*S*,8*R*,9*R*)-2-pentoxyclovan-9-ol (10). 1 g (4.54 mmol) of

(−)-caryophyllene oxide was dissolved by stirring in 20 mL of either n-propanol or n-pentanol at room temperature. Then, 60 mg (0.47 mmol) of tetracyanoethylene (TCNE) were added and the reaction was monitored by TLC. After 18 h the solvent was evaporated under reduced pressure. Column chromatography of the crude reaction mixture on silica gel, eluted with a gradient mixture of petroleum ether–EtOAc, combined with HPLC purification, yielded compound **9** (301 mg, 1.07 mmol, 24%) or 284 mg of compound **10** (284 mg 0.92 mmol, 20%).

(1*S*,2*S*,5*S*,8*R*,9*R*)-2-Propoxyclovan-9-ol (9). Obtained as white crystals; mp 68–70 °C (from EtOAc); $[\alpha]_D^{25}$ +9.3 (*c* 0.38, $CHCl_3$); IR (film) ν_{max}/cm^{-1} 3447 (OH),;δ_H (400 MHz, $CDCl_3$) 0.85 (3H, s, H_3-13α), 0.90 (3H, t, *J* 6.8, H_3-3′), 0.95 (3H, s, H_3-15), 0.97 (1H, m, H-12a), 1.01 (3H, s, H_3-14β), 1.09 (1H, m, H-7a), 1.10 (1H, m, H-11a), 1.29 (1H, m, H-6a), 1.37 (1H, m, H-7b), 1.39 (1H, m, H-5β), 1.39 (1H, m, H-6b), 1.49 (1H, dd, *J* 10.4 and 11.8, H-3β), 1.55 (2H, m, H-2′), 1.57 (1H, m, H-12b), 1.58 (1H, m, H-10α), 1.66 (1H, dd, *J* 6.0 and 11.8, H-3α), 1.69 (1H, m, H-11b), 1.97 (1H, dddd, *J* = 3.2; 4.8; 14.2; 14.3, H-10β), 3.31 (1H, m, H-9β), 3.36 (1H, m, H-1′a), 3.39 (1H, dd, *J* 6.0 and 10.4, H-2α), 3.42 (1H, m, H-1′b); δ_C (100 MHz, $CDCl_3$) 10.7 (q, C-3′), 20.6 (t, C-6), 23.4 (t, C-2′), 25.4 (q, C-13α), 26.1 (t, C-10), 26.7 (t, C-11), 28.4 (q, C-15), 31.3 (q, C-14β), 33.2 (t, C-7), 34.7 (s, C-8), 36.5 (t, C-12), 37.0 (s, C-4), 44.3 (s, C-1), 44.7 (t, C-3), 50.6 (d, C-5), 72.3 (t, C-1′), 75.3 (d, C-9), 88.3 (d, C-2); HMBC (selected correlations) C-1 → H-2α; C-2 → H-3α, H-3β, H-12a, H-12b; C-1′ → H_2-2′, H_3-3′; C-2′ → H-1′a. H-1′b, H_3-3′; C-3′ → H-2′, H-1′a. H-1′b; EIMS *m/z* 280 (M^+, 16%), 265 (M^+ − 15, 12), 220 (M^+ − 60, 12), 205 (21), 187 (13), 161 (21), 127 (100), 105 (27), 85 (73); HREIMS *m/z* 280.2422 $[M]^+$ (calcd for $C_{18}H_{32}O_2$, 280.4455).

(1*S*,2*S*,5*S*,8*R*,9*R*)-2-Pentoxyclovan-9-ol (10). Obtained as white crystals; mp 35–38 °C (from EtOAc); $[\alpha]_D^{25}$ +11.3 (*c* 0.36, $CHCl_3$); IR (film) ν_{max}/cm^{-1} 3443 (OH); δ_H (400 MHz, $CDCl_3$) 3.37 (1H, dd, *J* 5.6, 10.0, H-2α), 1.48 (1H, dd, *J* 10.0, 11.9, H-3β), 1.66 (1H, dd, *J* 5.6, 11.9, H-3α), 1.38 (1H, m, H-5β), 1.28–1.42 (2H, H-6a, H-6b), 1.10 (1H, m, H-7a), 1.38 (1H, m, H-7b), 3.30 (1H, m, H-9β), 1.59 (1H, m, H-10α), 1.97 (1H, dddd, *J* 3.2, 5.0, 14.1, 14.2, H-10β), 1.10 (1H, m, H-11a), 1.70 (1H, m, H-11b), 0.97 (1H, m, H-12a), 1.58 (1H, d, *J* 12.8, H-12′), 0.84 (3H, s, H_3-13α), 1.01 (3H, s, H_3-14β), 0.95 (3H, s, H_3-15), 3.42 (2H, m, H_2-1′), 1.53 (2H, m, H_2-2′), 1.28–1.33 (4H, H_2-3′, H_2-4′), 0.89 (3 H, t, *J* 7.0) δ_C (100 MHz, $CDCl_3$) 14.1 (q, C-5′), 20.6 (t, C-6), 22.6 (t, C-4′), 25.4 (q, C-13), 26.1 (t, C-10), 26.7 (t, C-11), 28.4 (q, C-15), 28.4 (t, C-3′), 29.9 (t, C-2′), 31.3 (q, C-14β), 33.2 (t, C-7), 34.7 (s, C-8), 36.5 (t, C-12), 37.0 (s, C-4), 44.3 (s, C-1), 44.7 (t, C-3), 50.55 (d, C-5), 70.6 (t, C-1′), 75.2 (d, C-9), 88.3 (d, C-2); HMBC (selected correlations) C-2 → H-3α, H-3β, H-12a, H-12b, H_2-1′; C-1′ → H-2α, H_2-2′; C-4′ → H_2-2′, H_3-5′; C-5′ → H_2-4′; EIMS *m/z* 308 (M^+, 14%), 293 (M^+ − 15, 10), 252 (8), 234 (18), 220 (22), 203 (74), 187 (32), 155 (100), 135 (40), 121 (48), 95 (50), 85 (80); HREIMS *m/z* 308.2662 $[M]^+$ (calcd for $C_{20}H_{36}O_2$, 308.4986).

Fungal cultures

P. palustris was isolated from a propolis sample collected in Minas Gerais State (Brazil) and kept in water–glycerol 80% at −40 °C. This strain is on deposit at the Biotechnology and Bioassay Laboratory at the Universidade Federal de Minas Gerais (Brazil).

The *P. minioluteum* culture employed in this work was isolated from Serra do Cipó soil (Minas Gerais, Brazil) and kept in water–glycerol 80% at −40 °C. This strain is on deposit at the Biotechnology and Bioassay Laboratory of the Universidade Federal de Minas Gerais (Brazil).

General culture conditions

P. palustris was grown on a medium composed of malt extract 30 g L^{-1} dissolved in water. *P. minioluteum* was grown on a medium composed of glucose (10 g L^{-1}), peptone (2.5 g L^{-1}), NaCl (2.5 g L^{-1}), K_2HPO_4 (0.5 g L^{-1}) and $MgSO_4 \cdot 7H_2O$ (0.25 g L^{-1}). Surface cultures were grown in Roux bottles containing 150 mL of the liquid medium. Cultures were stirred in 500 mL conical flasks containing 250 mL of liquid medium. The substrate dissolved in ethanol was added to each flask and the fermentation continued for an additional period of time (see below). The mycelium was filtered and washed with EtOAc. The broth was saturated with sodium chloride and extracted with EtOAc. The extracts were dried over sodium sulfate, the solvent was evaporated and the residues chromatographed on silica gel in a gradient mixture of petroleum ether and EtOAc of increasing polarity. In every biotransformation, a control containing the substrate and the culture medium, without the presence of the micro-organism, was run in parallel to assess substrate stability in the culture medium. Moreover, a negative control containing only the culture medium and the fungus was prepared to assess micro-organism secondary metabolite production to make certain that the isolated compounds were biotransformation products and not natural metabolites.

Biotransformation of (1*S*,2*S*,5*S*,8*R*,9*R*)-2-methoxyclovan-9-ol (1) by *P. palustris*. Compound **1** (120 mg) was distributed between 8 Roux bottles containing a 4-day culture of *P. palustris* and grown for a further 10 days. Column chromatography, followed by semi-preparative HPLC purification, yielded **3** (3.4 mg, 3%) and **4** (11.0 mg, 10%), **2** (2.3 mg, 2%), **5** (1.1 mg, 1%), and **6** (4.8 mg, 4%).

(1*R*,2*S*,5*S*,8*R*,9*S*,10*R*)-2-Methoxyclovane-9,10-diol (5). Obtained as a colourless oil; $[\alpha]_D^{25}$ +4.5 (*c* 0.10 in MeOH); IR: ν_{max}/cm^{-1} 3420 (OH); ^1H and ^{13}C-NMR data see Table 1; HMBC (selected correlations) C1 → H-3α; C4 → H-3α,H_3-13α,H_3-14β; C5 → H-3α,H-11α,H_3-13α,H_3-14β; C9 → H_3-15; C-10 → H-11α, H-11β; EIMS *m/z* 268 $[M]^+$ (6), 250 (3), 236 (15), 218 (8), 205 (10), 192 (14), 161 (22), 137 (50), 99 (100); HRMS *m/z* 268.2024 $[M]^+$ (calcd for $C_{16}H_{28}O_3$, 268.2038).

(1*S*,2*S*,5*S*,7*R*,8*R*,9*R*)-2-Methoxyclovane-7,9-diol (6). Obtained as colourless needles; mp 77–80 °C (from EtOAc); $[\alpha]_D^{25}$−11.5 (*c* 0.11 in, MeOH); IR: ν_{max}/cm^{-1} 3410 (OH); ^1H and ^{13}C-NMR data see Table 1; HMBC (selected correlations) C1 → H-11a, H-11b, H-12a,H-12b; C2 → –OC$\underline{H_3}$,H-3α,H-3β, H-11a,H-11b

H-12a,H-12b; C5 → H-12a,H_3-13α,H_3-14β; C7 → H-6α,H-6β, H-12a,H-12b,H_3-15; C-8 → H-6α,H_3-15; HRMS *m/z* 268.1979 $[M]^+$ (calcd for $C_{16}H_{28}O_3$, 268.2038).

Biotransformation of (1*S*,2*S*,5*S*,8*R*,9*R*)-2-methoxyclovan-9-ol (1) by *P. minioluteum*. Compound **1** (450 mg) was distributed between 15 Roux bottles containing a 4-day culture of *P. minioluteum* and grown for a further 10 days. Ethyl acetate extraction, solvent removal *in vacuo*, followed by column chromatography and semi-preparative HPLC purification, yielded compounds **3** (73 mg, 0.30 mmol, 17%) and **4** (48 mg, 0.20 mmol, 11%).

Biotransformation of (1*S*,2*S*,5*S*,8*R*,9*R*)-2-propoxyclovan-9-ol (9) by *P. minioluteum*. Compound **9** (60 mg) was distributed between 2 Roux bottles containing a 4-day culture of *P. minioluteum* and grown for a further 10 days. Ethyl acetate extraction, solvent removal *in vacuo*, followed by column chromatography and semi-preparative HPLC purification, yielded compounds **3** (4 mg, 0.017 mmol, 8%) and **4** (1 mg, 0.004 mmol, 2%) and allowed recuperation of 7 mg of compound **9**.

Biotransformations of (1*S*,2*S*,5*S*,8*R*,9*R*)-2-propoxyclovan-9-ol (10) and (1*S*,2*S*,5*S*,8*R*,9*R*)-2-(2′-(*p*-nitrophenoxy)ethoxy)clovan-9-ol (11) by *P. minioluteum*. Compounds **10** and **11** (60 mg each) were independently distributed between 2 Roux bottles containing a 4-day culture of *P. minioluteum* and grown for a further 10 days. Ethyl acetate extraction, solvent removal *in vacuo*, followed by column chromatography, permitted recovery of starting material. No clovane derivatives were obtained.

Biotransformation of (1*S*,2*S*,5*S*,8*R*)-8,9-seco-8-hydroxy-2-methoxyclovan-9-olactone (8) by *P. minioluteum*. Lactone **8** (240 mg) was distributed between 8 Roux bottles containing a 4-day culture of *P. minioluteum* and grown for a further 10 days. Ethyl acetate extraction, solvent removal *in vacuo*, followed by column chromatography and semi-preparative HPLC purification, yielded compound **2** (37 mg, 0.15 mmol, 16%).

(1*S*,2*S*,5*S*,8*R*)-Rumphellclovane A (2). Colourless oil, $[\alpha]_D^{25}$ +6.2 (*c* 0.10, MeOH); (lit.,[10] $[\alpha]_D^{23}$ +11 (*c* 0.025, $CHCl_3$)).

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