Structure−Activity Relationships in the Fungistatic Activity against *Botrytis cinerea* of Clovanes Modified on Ring C

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The preparation of clovanes **4**, **5**, **6**, **8**, and **9**, which bear different levels of oxidation on ring C, is described for the first time. The biotransformation of compounds **5**, **6**, and **9** by the fungus *Botrytis cinerea* is investigated, yielding compounds **10**, **11**, and **12**, which are described for the first time, together with compounds **4**−**6**, **8**, and **9**. The evaluation of the fungistatic activity against *B. cinerea* of compounds **6**, **9**, **12**, **18**, **19**, **20**, and **21** is reported. Comparison of these results with previously published data shows first that the inclusion of hydroxyl groups on ring C leads to a decrease in the biological activity and, second, that the presence of a 9α-hydroxyl group and an alkyl chain at C-2 plays an important role in the fungistatic activity against *B. cinerea* of compounds with a clovane skeleton.

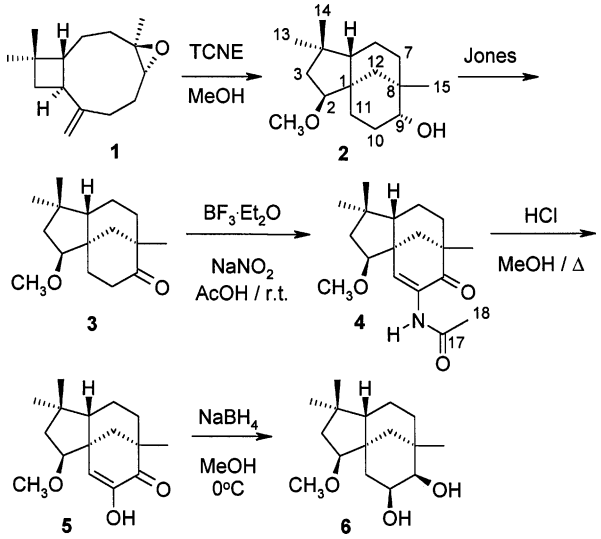
The fungus *Botrytis cinerea* is a serious plant pathogen[1] that has developed resistance to some commercial fungicides.[2] In addition, some fungicides, such as dicarboximide and procymidone, are persistent enough to be detected in vegetables[3] and soil[4] and in grapes, must, and wine, even several weeks after vinification.[5] Recently, it has been shown that procymidone has effects similar to flutamide on the sexual differentiation in mice,[6] and also it causes hepatotoxic disorders in fish.[4] Consequently, there is interest in the development of novel rationally designed antifungal agents with activity against *B. cinerea*. We have shown[7] that 2*β*-methoxyclovan-9α-ol (**2**) is an active fungistatic agent against *B. cinerea*. This botryane mimic is readily available by cyclization and rearrangement of caryophyllene oxide (**1**).[8] Its detoxification by this fungus has also been studied,[9] and this suggests that active products with the clovane skeleton should not persist for long after their application.

The development of this class of compounds as fungistatic agents against *B. cinerea* required the evaluation of a number of derivatives with clovane skeleton possessing different patterns of oxidation. In this paper we report the preparation of clovane derivatives with different levels of oxidation on ring C, which is topologically coincident with the region of botryane precursors which undergoes an oxidative cleavage to yield botrydial, dihydrobotrydial (**7**),[10] and derivatives.[11] We also describe the metabolism of these clovanes by *B. cinerea* and the evaluation of their biological activity against *B. cinerea* in comparison with that of other clovanes.

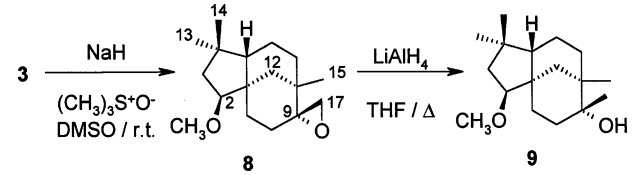
Results and Discussion

The chemical transformations that were carried out on the clovanes are summarized in Schemes 1 and 2. 2*β*-Methoxyclovan-9α-ol (**2**), obtained from caryophyllene oxide (**1**) by cyclization and rearrangement with TCNE in methanol,[8] was treated with Jones' reagent[12] to yield 2*β*-methoxyclovan-9-one (**3**) in 92% yield.[9] This ketone was treated with sodium nitrite and boron trifluoride etherate in acetic acid to give compound **4**, in 35% yield (Scheme 1). The ^{1}H NMR spectrum of this product showed signals at δ_H 7.81 (1H, br s), 8.09 (1H, d, $J = 2.1$ Hz), and 2.1 ppm (3H, s), which were assigned to an amide proton, an olefinic proton, and the methyl group of an acetamide, respectively. The ^{13}C NMR spectrum revealed a carbonyl resonance at δ_C 168.6 ppm and alkene resonances at δ_C 135.3 and 131.3 ppm. The high-resolution mass spectrum (*m*/*z* 305.1991 $[M]^+$) was consistent with the formula $C_{18}H_{27}NO_3$. The structure for this compound was established by X-ray crystal diffraction, which showed that it was *N*-(2*β*-methoxy-9-oxoclov-10-enyl) acetamide (**4**) (Figure 1).

Scheme 1



Scheme 2



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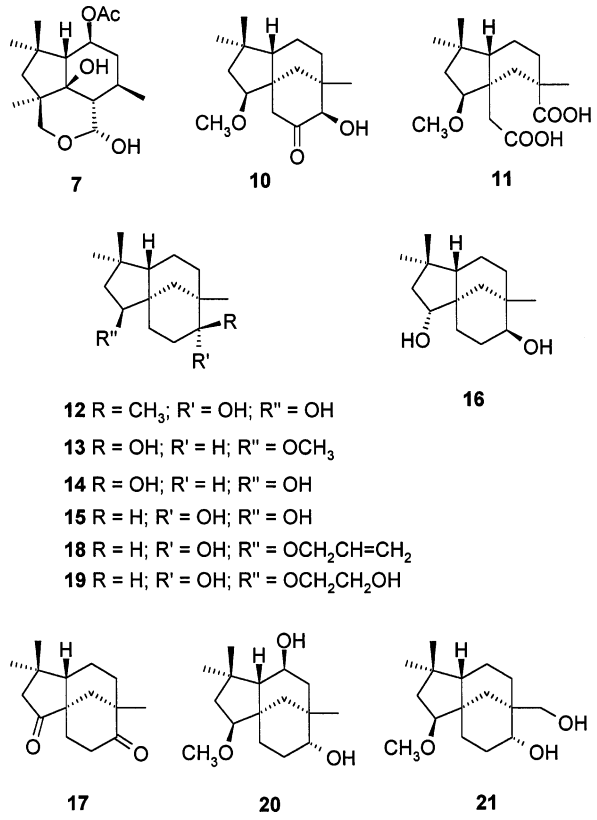
Scheme 3



Hydrolysis of the acetamide **4** with dilute hydrochloric acid in methanol gave 10-hydroxy-2β-methoxyclovan-10-en-9-one (**5**) in 68% yield (Scheme 1). The IR spectrum of this product contained absorptions at 3429 and 1668 cm^{-1}, while the ^{1}H NMR spectrum contained an alkene resonance [δ_H 6.52 (lH, d, J = 2.0 Hz)] which was assigned to H-11. Reduction of compound **5** with sodium borohydride in dry methanol gave 2β-methoxyclovan-9β,10β-diol (**6**) in 91% yield (Scheme 1). The IR spectrum contained a hydroxyl absorption (3410 cm^{-1}), while the ^{1}H NMR spectrum lacked the alkene resonance, but contained two new methine resonances [δ_H 3.23 (1H, dd, J = 6.7, 4.9 Hz) and δ_H 4.17 (1H, d, J = 4.9 Hz)] attributed to secondary alcohols. These were assigned to H-10α and H-9α, respectively. The stereochemistry of the reduction was established by NOE studies. Irradiation of the signal at δ_H 3.23 not only enhanced the signal at δ_H 4.17 (4.3%) but also enhanced a signal at δ_H 1.14 (3.5%) (1H, d, J = 13 Hz), which was assigned to one of the protons at C-12. Hence H-10 was on the α face of the clovane.

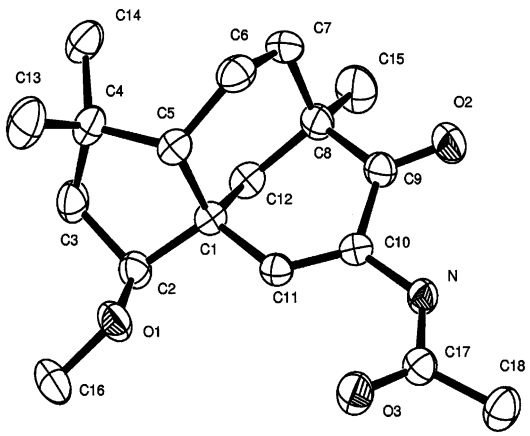


Figure 1. X-ray structure of compound **4** drawn by ORTEP.

Table 1. Metabolites of Clovanes by *B. cinerea*

substrate	metabolites
5	**10**, **11**, **7**
6	**10**, **7**
9	**12**, **7**

The methoxyketone **3** was converted to the exocyclic epoxide **8** in 76% yield by a Corey−Chaykovsky reaction[13] using freshly prepared trimethylsulfoxonium iodide.[14] This reaction introduces a methylene group with an equatorial conformation. The product possessed an IR absorption at 916 cm^{-1} and NMR signals [δ_H 2.29 and 2.70 ppm (each 1H, d, J = 4.5 Hz (H-17)), δ_C 62.92 ppm (s, C-9) and 49.45 ppm (t, C-17)], confirming the structure as 9α,17-epoxy-2β-methoxyclovane (**8**).

Reduction of the epoxide **8** with lithium aluminiun hydride in THF gave the C-9 tertiary alcohol **9** in 94% yield (Scheme 2). The ^{1}H NMR spectrum showed singlets at δ_H 3.28, 0.80, 0.86, 0.96, and 1.05 ppm (each 3H, s), corresponding to a methoxy group and four methyl groups. The presence of IR absorption at 3645 cm^{-1} and a resonance at δ_C 74.06 ppm (s) confirmed the presence of a tertiary alcohol. Hence the reduction product had the structure 2β-methoxy-9β-methylclovan-9α-ol (**9**).

The substrates 10-hydroxy-2β-methoxyclov-10-en-9-one (**5**), 2β-methoxyclovan-9β,10β-diol (**6**), and 2β-methoxy-9β-methylclovan-9α-ol (**9**) were each incubated separately with *B. cinerea* for 7−10 days on a shaken culture. In every culture, a certain amount of the characteristic metabolite of *B. cinerea*, dihydrobotryidial (**7**), is produced. The separate feeding of compounds **5**, **6**, and **9** to liquid cultures of *B. cinerea* did not suppress the production of botryane metabolites, at the given levels. The metabolites that were isolated have been tabulated (see Table 1). Their structures were established as follows.

Compound **10** had IR absorptions at 3408 and 1708 cm^{-1}, which were consistent with the presence of an α-hydroxy ketone. The ^{1}H NMR spectrum showed signals at δ_H 3.67 (1H, d, J = 1.1 Hz, C*H*OH), 2.33 (1H, dd, J = 14.4, 3.3 Hz, C*H*HCO), and 2.44 (1H, dd, J = 14.4, 1.5 Hz, CH*H*CO), which were assigned to H-9α, and to H-11 and H-11′, respectively. An NOE effect that was observed between the signals at δ_H 3.67 (H-9α) and 1.60 (1H, ddd, J = 13.6, 1.0, 1.0 Hz, H-12) supported the assignment of the β-stereochemistry to the hydroxyl group at C-9. The ^{13}C NMR spectrum showed resonances at δ_C 82.3 and 212.6 ppm, consistent with the structure 9β-hydroxy-2β-methoxyclovan-10-one (**10**) for the metabolite.

Compound **11** possessed ^{1}H NMR signals at δ_H 3.03 and 3.10 (each 1H, d, J = 17.4 Hz), which were assigned to H-11 and H-11′. In the ^{13}C NMR spectrum there were signals at δ_C 181.1 and 174.6 ppm, which were assigned to carboxyl groups at C-9 and C-10. The structure of this metabolite was established by X-ray crystallography as 2β-methoxy-9,10-secoclovan-9,l0-dioic acid (**11**) (Figure 2).

The ^{1}H NMR spectrum of compound **12** lacked a methoxy signal, but it contained a signal at δ_H 3.80 ppm (lH, dd, J = 9.5, 5.7 Hz), which was assigned to H-2α. The spectrum retained four methyl group signals at δ_H 0.88, 0.93, 1.04, and 1.13 ppm, while the ^{13}C NMR spectrum contained a tertiary alcohol signal at δ_C 74.28 ppm, which was assigned to C-9. The structure of this metabolite was established by X-ray crystallography as 9β-methylclovane-2β,9α-diol (**12**) (Figure 3), and it allowed, in turn, the confirmation of the stereochemistry assigned to the parent compounds **8** and **9**.

In the biotransformation of the clovanes, the methoxyl group appears to be metabolized depending upon the

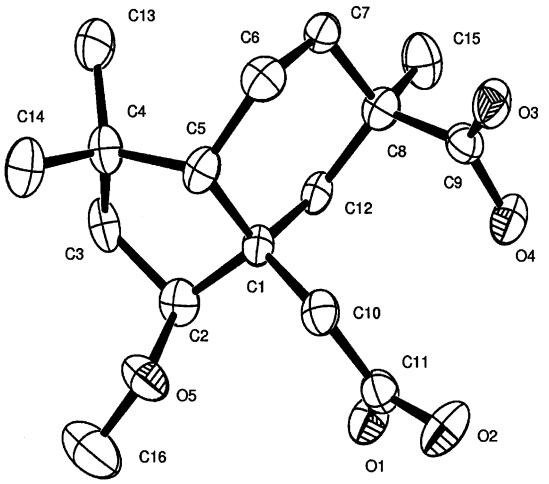


Figure 2. X-ray structure of compound **11** drawn by ORTEP.

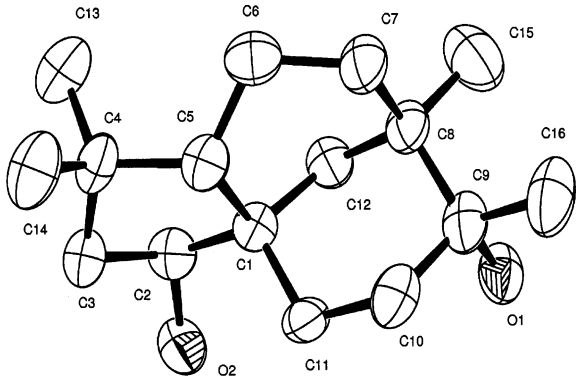


Figure 3. X-ray structure of compound **12** drawn by ORTEP.

number of hydroxyl groups present in the substrate. It did not take place when there were two hydroxyl groups or a hydroxyl and a carbonyl group in the substrate. The reduction of the carbonyl group at C-9 in the biotransformation of compound **5** to compound **10** follows the Prelog rule for the asymmetric reduction of ketones.[15]

The antifungal properties of compounds **6**, **9**, and **12** were determined against the growth of *B. cinerea* using the poisoned food technique.[16] The commercial fungicide Euparen was used as a standard for comparison in this test. Several levels of inhibition were observed. We also report here the antifungal properties of compounds **18**, **19**,[8] **20**,[17] and **21**,[7] which will be used in the discussion of the structure−activity relationships of clovanes versus *B. cinerea*, together with already published values for compounds **2**, **3**, **13**, **14**, **15**, **16**, and **17**.[9]

As shown in Figure 4 we observed a general decrease in the activity of compounds with the clovane skeleton when a second hydroxyl group was added to the structure even if an ether group is present at C-2. Furthermore, the closer the hydroxyl group is to position C-9 in the clovane skeleton, the lower is the activity of the compound, which decreases in the order **2** > **19** > **20** > **21** > **6**.

One of the detoxification mechanisms of 2β-methoxyclovan-9α-ol (**2**) is the oxidation and epimerization of the OH group at C-9.[9] Therefore, if we can prevent this oxidation or epimerization, the activity might be prolonged. Compound **9** does not undergo biotransformation at C-9. However the biological activity of compounds **9** and **12** was less than that of the equivalent compounds (compounds **2** and **15**) which lack the methyl group at C-9 (Figure 5).

The following conclusions can be drawn from this study of structure−activity relationships. First, when compared

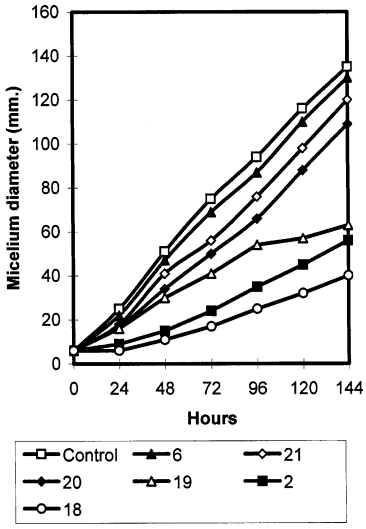


Figure 4. Comparison of fungal growth inhibition (*B. cinerea)* among compounds **2**, **6**, **18**, **19**, **20**, and **21** (100 ppm dose).

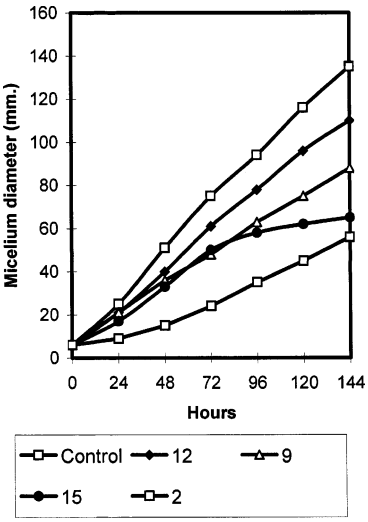


Figure 5. Comparison of fungal growth inhibition (*B. cinerea)* among compounds **2**, **9**, **12**, and **15** (100 ppm dose).

to 2β-methoxyclovan-9α-ol (**2**), the inclusion of additional hydroxyl groups, especially on ring C, leads to a decrease in biological activity. Second, the presence of a 9α-hydroxyl group is important for biological activity. Third, the introduction of an alkyl chain at C-2 can significantly increase the potency of this class of compounds as inhibitors of the plant pathogen *B. cinerea*.[18]

Experimental Section

General Experimental Procedures. Melting points were measured with a Reichert-Jung Kofler block and are uncorrected. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 881 spectrophotometer. ^{1}H and ^{13}C NMR measurements were obtained on Bruker 300 DPX and Bruker 500 AMX NMR spectrometers with $SiMe_4$ as internal reference. 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 VG 12-250 spectrometer at 70 eV. HPLC was performed with a Hitachi/Merck L-6270 apparatus equipped with a UV−vis detector (L 4250) and a differential refractometer detector (RI-71). TLC was performed on Merck Kiesegel 60 F_{254}, 0.2 mm thick. Silica gel (Merck) was used for column chromatography. Purification by HPLC was accomplished using a Si gel

Table 2. ^{13}C NMR Data (75.5 MHz, $CDCl_3$) for Compounds **4**−**6**, **8**−**11**, and **12**

carbon[a]	2	3	4	5	6	8	9	10	11	14
1	†44.1 s	†44.8 s	†41.7 s	†41.6 s	†43.0 s	†44.3 s	†44.1 s	†49.3 s	†40.7 s	†44.3 s
2	90.1 d	89.1 d	87.3 d	87.3 d	89.8 d	89.5 d	89.6 d	88.3 d	88.2 d	80.6 d
3	44.0 t	44.0 t	44.0 t	43.9 t	43.8 t	41.6 t	38.4 t	43.5 t	38.8 t	37.3 t
4	†37.0 s	†44.4 s	†38.9 s	†39.2 s	†37.4 s	†37.1 s	†37.2 s	†38.6 s	†48.9 s	†37.3 s
5	50.6 d	50.9 d	45.4 d	45.6 d	49.7 d	58.0 d	57.9 d	50.7 d	51.5 d	50.8 d
6	20.5 t	‡20.5 t	‡18.3 t	‡18.2 t	‡20.1 t	‡20.4 t	‡20.9 t	‡18.5 t	‡19.9 t	‡21.1 t
7	33.1 t	‡32.5 t	‡33.4 t	‡33.5 t	‡27.1 t	‡32.8 t	‡33.0 t	‡28.7 t	‡31.3 t	‡32.7 t
8	†34.7 s	†38.3 s	†48.5 s	†48.1 s	†34.6 s	†32.9 s	†37.0 s	†39.8 s	†39.8 s	†37.7 s
9	75.2 d	216.3 s	200.4 s	210.6 s	78.1 d	62.9 s	74.1 s	82.3 d	181.1 s	74.3 s
10	26.0 t	35.7 t	131.3 s	146.5 s	70.2 d	27.8 t	28.5 t	212.6 s	174.6 s	28.4 t
11	26.5 t	‡34.0 t	135.3 d	122.8 d	43.1 t	‡31.1 t	‡32.5 t	47.4 t	43.5 t	‡33.1 t
12	36.5 t	‡42.7 t	33.4 t	41.7 t	38.1 t	‡43.9 t	‡43.8 t	41.5 t	37.8 t	‡47.6 t
13	25.4 q	26.2 q	24.6 q	25.2 q	25.7 q	25.4 q	25.4 q	26.5 q	28.8 q	24.8 q
14	31.2 q	32.0 q	33.4 q	33.7 q	31.4 q	24.0 q	23.8 q	32.1 q	30.8 q	24.0 q
15	28.4 q	24.9 q	28.4 q	28.5 q	30.1 q	31.2 q	31.3 q	28.3 q	34.4 q	31.6 q
16	58.2 q	57.7 q	57.0 q	55.8 q	58.0 q	50.4 q	50.9 q	57.3 q	56.5 q	
17			168.6 s			49.4 t	24.8 q			24.8 q
18			25.6 q							

[a] Notation for attached protons: s = C; d = CH; t = CH_2; q = CH_3; †, ‡ interchangeable signals.

column (Hibar 60, 7 μm, 1 cm wide, 25 cm long). X-ray diffraction data collection was carried out on a Nonius Kappa CCD area detector diffractometer. Structure was determined using the WinGX software package[19] and the SHELXL-97 program.[20] ORTEP[21] was used to generate Figures 1, 2, and 3.

Microorganism and Antifungal Assays. The culture of *Botrytis cinerea* employed in this work, *B. cinerea* (UCA 992), was obtained from grapes from the Domecq vineyard, Jerez de la Frontera, Cádiz, Spain. This culture of *B. cinerea* has been deposited at the Mycological Herbarium Collection (UCA), Facultad de Ciencias, Universidad de Cádiz. Bioassays were performed by measuring inhibition of radial growth on agar medium in a Petri dish. Test compounds were dissolved in EtOH to give a final compound concentration in the culture medium of 50−200 mg L^{-1}. Solutions of test compounds were added to glucose-malt-peptone-agar medium (61 g of glucose-malt-peptone-agar per L, pH 6.5−7.0). The final EtOH concentration was identical in both the control and treated cultures. The medium was poured in 6 or 9 cm diameter sterile plastic Petri dishes, and a 5 mm diameter mycelial disk of *B. cinerea* cut from an actively growing culture was placed in the center of the agar plate. Inhibition of radial growth was measured for 6 days. Every concentration was evaluated in triplicate.

General Culture Conditions. *B. cinerea* (UCA 992) was grown on shaken cultures in a 250 mL conical flask at 25 °C for 3 days on Czapek-Dox medium (70 mL per flask) comprising (per L of distilled water) glucose (40 g), yeast extract (1 g), potassium dihydrogen phosphate (5 g), sodium nitrate (2 g), magnesium sulfate (0.5 g), ferrous sulfate (10 mg), and zinc sulfate (5 mg). The substrate dissolved in EtOH was added to each flask, and the fermentation continued for a further period (see below). The mycelium was filtered and washed with brine and EtOAc. The broth was saturated with NaCl, acidified (pH 2), and extracted with EtOAc. The extracts were separated into acidic and neutral fractions with aqueous sodium hydrogen carbonate. The acid fraction was recovered in EtOAc. The extracts were dried over Na_2SO_4, the solvent was evaporated, and the residues were chromatographed on silica gel in a gradient mixture of petroleum ether−EtOAc of increasing polarity. The acidic fractions were methylated with diazomethane prior to chromatography. Chromatography of the acidic fractions yielded inseparable mixtures.

***N*-(2*β*-Methoxy-9-oxoclov-10-enyl) Acetamide (4).** Boron trifluoride complex (18 mL, 0.15 mol, 26.5 equiv, in diethyl ether) was added dropwise to a magnetically stirred solution of 2*β*-methoxyclovan-9-one (**3**) (1.4. g, 5.5 mmol, 1 equiv) in acetic acid (46 mL) under nitrogen, at room temperature. $NaNO_2$ (7.6 g, 0.11 mol, 20 equiv) was added in portions over 3 h. After an additional 2 h, boron trifluoride (18 mL, 0.15 mol, 26.5 equiv, in diethyl ether) was added, and the solution was stirred for 3 h and then quenched by careful addition of H_2O. The crude reaction mixture was extracted with CH_2Cl_2 and washed with H_2O, and the organic phase was dried over anhydrous Na_2SO_4. The solvent was removed in vacuo to leave a yellow oil, which was purified by column chromatography on silica gel using (3:7) EtOAc in light petroleum as eluent to give *N*-(2*β*-methoxy-9-oxoclov-10-enyl) acetamide (**4**) (600 mg, 35%) as white needles (EtOAc): mp 119−121°C; IR (Nujol) ν_{max} 3293, 1666, 1623 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.09 (1H, d, J = 2.1 Hz, H-11), 7.81 (1H, br s, N*H*), 3.39 (1H, t, J = 10.0, 3.6 Hz, H-2α), 3.35 (3H, s, OCH_3), 2.10 (3H, s, HNCOCH_3), 1.85 (2H, s, CH_2C(CH_3)$_2$), 1.13 (3H, s, H-14), 1.09 (3H, s, H-15), 1.05 (3H, s, H-13); ^{13}C NMR (75.5 MHz, $CDCl_3$) (see Table 2); HREIMS m/z 305.1992 $[M]^+$ (calcd for $C_{18}H_{27}NO_3$, 305.1991).

X-ray crystal structure of *N*-(2*β*-methoxy-9-oxoclov-10-enyl) acetamide (4): white crystal, obtained from EtOAc; $C_{18}H_{27}NO_3$; crystal size 0.4 × 0.4 × 0.4 mm. Cell parameters: a = 9.7008(5) Å, b = 12.1777(4) Å, c = 14.3201(8) Å, α = β = γ = 90°, V = 1691.7(1) Å^3, Z = 4, D_{calc} = 1.20 Mg/m^3, T = 173-(2) K, λ = 0.71073 Å, space group = $P2_12_12_1$ (No. 19), absorption coefficient = 0.08 mm^{-1}, $F(000)$ = 664, refinement with 2960 reflections (2364 > 2σI) led to final R indices (all data): R1 = 0.0644, wR2 = 0.1084; and GOF = 1.017. Crystallographic data (excluding structure factors) for the structure of this compound have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

10-Hydroxy-2*β*-methoxyclovan-10-en-9-one (5). HCl (32 mL, 2 N) was added dropwise to a stirred solution of acetamide **4** (600 mg, 1.96 mmol, 1 equiv) in MeOH (150 mL), and the mixture was heated to reflux for 2 h. After cooling, H_2O was added and the reaction mixture was extracted with Et_2O. The organic phase was washed with H_2O and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to give 10-hydroxy-2*β*-methoxyclovan-10-en-9-one (**5**) (350 mg, 68%) as an oil: IR (Nujol) ν_{max} 3429,1668 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.52 (1H, d J = 2.0 Hz, H-11), 5.92 (1H, s, O*H*), 3.34 (1H, t, J = 9.8, 3.5 Hz, H-2α), 3.33 (3H, s, OCH_3), 1.15 (3H, s, H-14), 1.12 (3H, s, H-15), 1.07 (3H, s, H-13); ^{13}C NMR (75.5 MHz, $CDCl_3$) (see Table 2); HREIMS m/z 263.1664 $[M - H]^+$ (calcd for $C_{16}H_{23}O_3$, 263.1647).

2*β*-Methoxyclovane-9*β*,10*β*-diol (6). Sodium borohydride (230 mg, 6.1 mmol, 4.5 equiv) was added to a solution of compound **5** (350 mg, 1.3 mmol, 1 equiv) in dry MeOH (25 mL) at 0 °C, and the reaction was stirred for 30 min. HOAc (2 mL) was added, and the solvent was evaporated under reduced pressure. The residue was recovered in EtOAc, and the resulting organic phase was washed with aqueous Na_2CO_3, H_2O, and brine and dried over anhydrous Na_2SO_4. The solvent

was removed in vacuo to give 2β-methoxyclovane-9β,10β-diol (**6**) (320 mg, 91%) as an oil: IR (Nujol) ν_{max} 3410 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.17 (1H, d, *J* 4.9 Hz, H-9α), 3.23 (1H, dd, *J* 4.9, 6.7 Hz, H-10α), 3.33 (3H, s, OC*H*$_3$), 3.32 (1H, t, *J* = 9.9, 5.5 Hz, H-2α), 1.14 (1H, d, *J* = 13, H-12a), 1.05 (3H, s, H-15), 1.03 (3H, s, H-14), 0.86 (3H, s, H-13); ^{13}C NMR (75.5 MHz, $CDCl_3$) (see Table 2); HREIMS *m*/*z* 266.1871 [M − 2H]$^+$ (calcd for $C_{16}H_{26}O_3$, 266.1881).

9α,17-Epoxy-2β-methoxyclovane (8). Sodium hydride (120 mg, 60% dispersion in mineral oil) was washed three times with hexane, then dried in vacuo. Dry DMSO (40 mL) was added and the solution stirred at room temperature for 20 min under a nitrogen atmosphere. Freshly prepared trimethylsulfoxonium iodide[14] (620 mg), dissolved in dry DMSO (30 mL), was added and the solution stirred at room temperature for a further 20 min. 2β-Methoxyclovan-9-one (**3**) (350 mg), dissolved in dry DMSO (30 mL), was added, and the reaction was allowed to stir at room temperature. After 14 h, the solution was poured into H_2O (150 mL) and extracted three times with EtOAc. The combined organic layers were washed three times with brine and then dried over Na_2SO_4. The EtOAc was removed in vacuo, and the yellow oil that remained was adsorbed onto silica and purified by column chromatography over silica. Elution with (5:95) EtOAc in petroleum ether gave 9α,17-epoxy-2β-methoxyclovane (**8**) (282 mg) as a colorless oil: IR (Nujol) ν_{max} 2929, 916 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.26 (3H, s, OC*H*$_3$), 3.24 (1H, dd, *J* = 10.1, 5.6 Hz, H-2α), 2.70 (1H, d, *J* = 4.5 Hz, H-17′), 2.29 (1H, d, *J* = 4.5 Hz, H-17), 0.96 (3H, s, H-14), 0.80 (3H, s, H-15), 0.64 (3H, s, H-13); ^{13}C NMR (75.5 MHz, $CDCl_3$) (see Table 2); EIMS *m*/*z* 264 [M]$^+$ (61), 249 [M − 15]$^+$ (27), 232 [M − MeOH]$^+$ (100), 217 [M − 15 − MeOH]$^+$ (35), 204 (23), 191 (39); HREIMS *m*/*z* 264.2081 (calcd for $C_{17}H_{28}O_2$, 264.2089).

2β-Methoxy-9β-methylclovan-9α-ol (9). Compound **8** (260 mg) was taken up in dry THF (100 mL) and was added to $LiAlH_4$ (40 mg) under nitrogen atmosphere. The reaction was heated under reflux for 10 h and then cooled. The excess hydride was destroyed by careful addition of THF−H_2O (1:1). H_2O (100 mL) was then added, and the reaction mixture was extracted three times with EtOAc. The combined organic layers were washed three times with brine and then dried over Na_2SO_4. The ethyl acetate was removed in vacuo to afford 2β-methoxy-9β-methylclovan-9α-ol (**9**) (245 mg) as a colorless oil: IR (Nujol) ν_{max} 3456 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.28 (3H, s, OC*H*$_3$), 3.25 (1H, dd, *J* = 10.1, 5.6, H-2α), 1.05 (3H, s, H-17), 0.96 (3H, s, H-14), 0.86 (3H, s, H-15), 0.80 (3H, s, H-13); ^{13}C NMR (75.5 MHz, $CDCl_3$) (see Table 2); EIMS *m*/*z* 266 [M]$^+$ (9), 251 [M − 15]$^+$ (18), 234 [M − MeOH]$^+$ (23), 216 [M − 18 − MeOH]$^+$ (16), 201 [M − MeOH − 18 − 15]$^+$ (15), 195 (20), 176 (57), 164 (100); HREIMS *m*/*z* 266.2257 (calcd for $C_{17}H_{30}O_2$, 266.2245).

Biotransformation of 10-Hydroxy-2β-methoxyclovan-10-en-9-one (5) by *B. cinerea*. Compound **5** (200 mg) was distributed over 12 flasks of *B. cinerea* and the fermentation grown for a further 7 days. Chromatography of the neutral fraction with a gradient mixture of petroleum ether−EtOAc of increasing polarity gave 9β-hydroxy-2β-methoxyclovan-10-one (**10**) (30 mg), 2β-methoxy-9,10-secoclovan-9,l0-dioic acid (**11**) (15 mg), and dihydrobotrydial (**7**)[10] (8 mg).

Biotransformation of 2β-Methoxyclovan-9β,10β-diol (6) by *B. cinerea*. Compound **6** (220 mg) was distributed over 12 flasks of *B. cinerea* and the fermentation grown for a further 7 days. Chromatography of the neutral fraction with a gradient mixture of petroleum ether−EtOAc of increasing polarity gave 2β-methoxyclovan-9β,10β-diol (**6**) (20 mg), 9β-hydroxy-2β-methoxyclovan-10-one (**10**) (30 mg), and dihydrobotrydial (**7**) (7 mg).

Biotransformation of 2β-Methoxy-9β-methylclovan-9α-ol (9) by *B. cinerea*. Compound **9** (150 mg) was distributed over 42 flasks and the fermentation grown for a further 10 days. Chromatography of the neutral fraction with a gradient mixture of petroleum ether−EtOAc of increasing polarity gave 9β-methylclovane-2β,9α-diol (**14**) (38 mg) and dihydrobotrydial (**7**) (28 mg).

9β-Hydroxy-2β-methoxyclovan-10-one (10): oil; IR (Nujol) ν_{max} 3480, 1708 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.85 (3H, s, H-13α), 0.94 (3H, s, H-15), 1.07 (3H, s, H-14β), 2.33 (1H, dd, *J* = 3.3, 14.4 Hz, C*H*CO), 2.44 (1H, dd, *J* = 1.5, 14.4 Hz, C*H*CO), 3.24 (3H, s, OC*H*$_3$), 3.35 (1H, dd, *J* = 9.4, 5.4 Hz, H-2α); ^{13}C NMR ($CDCl_3$, 75.5 MHz) (see Table 2); HREIMS *m*/*z* 266.1876 [M]$^+$ (calcd for $C_{16}H_{26}O_3$, 266.1881).

2β-Methoxy-9,10-secoclovane-9,l0-dioic acid (11): white needles (EtOAc); mp 178−180°C; IR (Nujol) ν_{max} 1689 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.08 (3H, s, H-13α), 1.16 (3H, s, H-15), 1.36 (3H, s, H-14β), 3.03 (1H, d, *J* = 17.4 Hz, C*H*H-COOH),3.10 (1H, d, *J* = 17.4 Hz, CH*H*COOH), 3.29 [3H, s, OC*H*$_3$]; 3.31 [1H, m, H-2α]; ^{13}C NMR ($CDCl_3$, 75.5 MHz) (see Table 2); HREIMS *m*/*z* 280.1650 [M − 18]$^+$ (calcd for $C_{16}H_{24}O_4$ requires 280.1675).

X-ray crystal structure of 2β-methoxy-9,10-secoclovane-9,10-dioic acid (11): white crystal, obtained from EtOAc; $C_{16}H_{26}O_5$; crystal size 0.4 × 0.05 × 0.05 mm. Cell parameters: *a* = 6.2588(6) Å, *b* = 13.8493(15) Å, *c* = 22.6116-(18) Å, α = β = γ = 90°, *V* = 1960.0(3) Å^3, *Z* = 4, D_{calc} = 1.30 Mg/m^3, *T* = 173(2) K, λ = 0.71073 Å, space group = $P2_12_12_1$ (No. 19), absorption coefficient = 0.35 mm^{-1}, *F*(000) = 816, refinement with 2614 reflections (1959 > 2σ*I*) led to final *R* indices (all data): R1 = 0.101, wR2 = 0.167; and GOF = 1.035. Crystallographic data (excluding structure factors) for the structure of this compound have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

9β-Methylclovane-2β,9α-diol (12): colorless needles (acetone−petroleum ether); mp 148−150°C; IR (film) ν_{max} 3364 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.88 (3H, s, H-13α), 0.93 (3H, s, H-15), 1.04 (3H, s, H-14β), 1.04 (3H, s, H-17), 3.80 (1H, dd, *J* = 5.7, 9.5 Hz, H-2α); ^{13}C NMR ($CDCl_3$, 75.5 MHz) (see Table 2); HREIMS *m*/*z* 252.2067 (calcd for $C_{16}H_{28}O_2$, 252.2089).

X-ray crystal structure of 9β-methylclovane-2β,9α-diol (12): colorless crystal, obtained from acetone−petroleum ether; $C_{16}H_{28}O_2$; crystal size 0.3 × 0.1 × 0.1 mm. Cell parameters: *a* = 20.868(7) Å, *b* = 20.868(7) Å, *c* = 10.522(6) Å, α = β = 90°, γ = 120°, *V* = 3968(3) Å^3, *Z* = 9, D_{calc} = 0.95 Mg/m^3, *T* = 293-(2) K, λ = 1.5418 Å, space group = R_3 (No. 146), absorption coefficient = 0.47 mm^{-1}, *F*(000) = 1260, refinement with 1836 reflections (1316 > 2σ*I*) led to final *R* indices (all data): R1 = 0.111, wR2 = 0.189; and GOF = 1.117. Crystallographic data (excluding structure factors) for the structure of this compound have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

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