The Biotransformation of Some Clovanes by *Botrytis cinerea*

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The metabolism of the fungistatic agent 2β-methoxyclovan-9α-ol (**2**) by the fungus *Botrytis cinerea* has been investigated. Biotransformation of compound **2** yielded compounds **3**−**5**, **7**, and **9**. The major metabolites of compound **2** each show much reduced biological activity when compared with the parent compound. Also studied were the effects of *B. cinerea* on the metabolism of the related compounds 2β-methoxyclovan-9-one (**3**), 2β-methoxyclovan-9β-ol (**4**), and clovan-2,9-dione (**6**). Compounds **3**, **4**, **8**, and **9** are described for the first time.

The fungus *Botrytis cinerea* is a serious plant pathogen that has developed resistance to some commercial fungicides.[1,2] Consequently, there is interest in the development of novel antifungal agents with activity against this organism. We have shown that 2β-methoxyclovan-9α-ol (**2**), a readily available cyclization and rearrangement product of caryophyllene oxide (**1**),[3] is a moderately active fungistatic agent against *Botrytis cinerea*.[4] However, its action decreases with time as it is metabolized by the fungus. In this paper we report on the metabolism by *B. cinerea* of 2β-methoxyclovan-9α-ol (**2**) and the related compounds 2β-methoxyclovan-9-one (**3**), 2β-methoxyclovan-9β-ol (**4**), and clovan-2,9-dione (**6**).

Results and Discussion

2β-Methoxyclovan-9α-ol (**2**) was obtained from caryophyllene oxide (**1**) by cyclization and rearrangement with tetracyanoethylene in methanol.[3] Oxidation with Jones' reagent[5] of compound **2** yielded 2β-methoxyclovan-9-one (**3**), which showed an absorption at 1711 cm^{-1} in its IR spectrum and a resonance at δ216.4 (s) in its ^{13}C NMR spectrum, both consistent with a ketone. Reduction of this ketone with lithium aluminum hydride proceeded from the less-hindered face to give 2β-methoxyclovan-9β-ol (**4**) (Scheme 1). The stereochemistry of this compound was confirmed by several NOE experiments (Figure 1). Clovan-2β,9α-diol (**5**) was obtained from caryophyllene oxide (**1**) by treatment with acid,[6] and oxidized to the diketone (**6**) with pyridinium chlorochromate (Scheme 2). The product showed absorptions at 1735 and 1710 cm^{-1} in its IR spectrum and resonances at δ219.4 and 213.3 (s) in its ^{13}C NMR spectrum consistent with the presence of two keto groups in the molecule.

The substrates 2β-methoxyclovan-9α-ol (**2**), 2β-methoxyclovan-9-one (**3**), 2β-methoxyclovan-9β-ol (**4**), and clovan-2,9-dione (**6**) were each incubated separately with *B. cinerea* for 4−16 days on surface culture. The metabolites that were isolated have been tabulated (see Table 1).

The metabolites were identified by their ^{1}H and ^{13}C NMR spectra (see Table 2) and by comparison with authentic samples prepared chemically from 2β-methoxyclovan-9α-ol (**2**) and caryophyllene oxide (**1**). Clovan-2β,9β-diol (**7**) was obtained as a minor compound from the reaction of clovan-2,9-dione (**6**) with sodium borohydride (Scheme 2). A remarkable feature was the change in the ^{1}H NMR coupling constant in compounds with H-9α stereochemistry. The C*H*(OH) resonance of the 9α-alcohol **2** was a broad

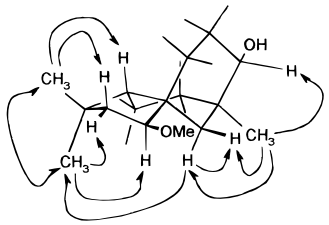
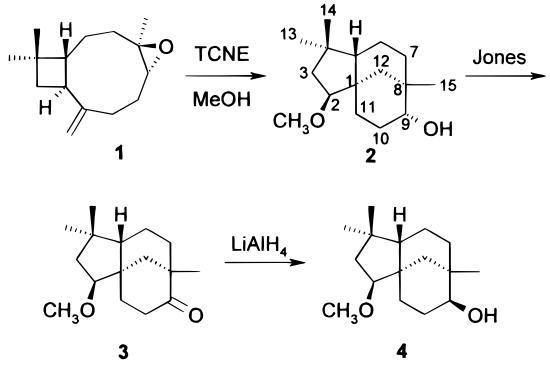


Figure 1. Selected NOE correlations observed for compound **4**.

Scheme 1



Scheme 2

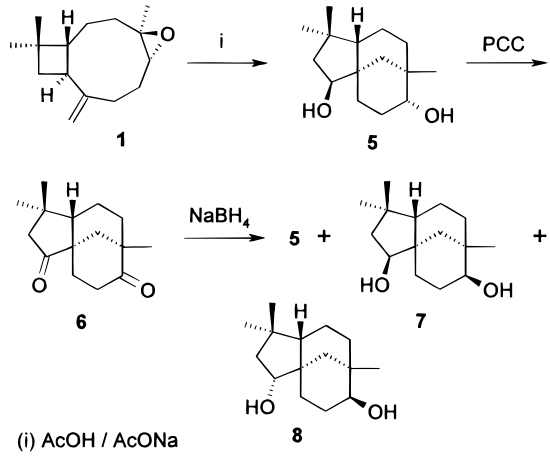


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Table 1. Metabolites of Clovanes by *B. cinerea*

substrate	metabolites
2[a]	**3**, **4**, **5**, **7**, **9**
3	**4**, **7**, **9**
4	**3**, **7**, **9**
6	*b*

[a] 4 days. [b] No clovane metabolites observed.

Table 2. ^{13}C NMR Data (50 MHz) for Compounds **3**, **4**, **6**−**9**

carbon	**3**[a]	**4**[a]	**6**[a]	**7**[a]	**8**[b]	**8**[a,c]	**9**[a]
1	44.8 s[d]	43.9 s[d]	48.9 s[d]	43.9 s[d]	45.9 s[d]	44.8 s[d]	42.4 s
2	89.1 d	89.36 d	220.1d	80.2 d	80.0 d	79.8 d	89.2 d
3	44.0 t	44.01 t	52.4 t	47.9 t	47.0 t	47.0 t	43.8 t
4	44.4 s[d]	37.05 s[d]	37.0 s[d]	37.3 s[d]	38.3 s[d]	37.9 s[d]	39.2 s
5	50.9 d	51.45 d	49.9d	51.3 d	52.6 d	51.4 d	52.0 d
6	20.5 t[e]	20.22 t	20.1 t	20.3 t[e]	23.1 t[e]	22.0 t[e]	19.9 t
7	32.5 t[e]	31.44 t	34.4 t	31.1 t[e]	38.9 t[e]	37.8 t[e]	30.6 t
8	38.3 s[d]	35.23 s[d]	43.9 s[d]	35.2 s[d]	35.0 s[d]	34.1 s[d]	31.0 s
9	216.3 d	78.09 d	214.0 d	78.0 d	78.7 d	78.0 d	77.9 d
10	35.7 t	27.82 t	36.2 t	27.8 t	30.6 t	29.6 t	22.5 t
11	34.0 t[e]	27.17 t	35.2 t	27.2 t	28.6 t[e]	27.4 t[e]	27.4 t
12	42.7 t[e]	42.90 t	39.1 t	42.0 t	36.5 t	35.2 t	36.9 t
13	26.2 q	25.42 q	24.9 q[e]	25.5 q	26.2 q[f]	25.8 q[f]	73.5 t
14	32.0 q	31.32 q	30.6 q	31.5 q	32.8 q[f]	32.4 q[f]	25.4 q
15	24.9 q	28.72 q	24.8 q[e]	28.7 q	30.1q	29.3 q	31.2 q
1′	57.7 q	58.10 q					58.2 q

[a] In $CDCl_3$. [b] In CD_3OD. [c] Assignations based on those of CD_3OD spectrum. [d −f] Interchangeable signals.

singlet (δ_H 3.30 H-9β), and the C*H*(OH) resonance of the 9β-alcohols (e.g., **4**) was a double doublet (δ_H 3.19, J = 5.1, 10.5 Hz, H-9α). The methyl group that had been hydroxylated in compound **9** was identified by changes in the ^{13}C NMR spectrum (see Table 2) and by a NOE effect between one of the −CH_2OH signals (δ_H 3.46 ppm) and H-5β(δ_H 1.15, dd, J = 3.0, 12.7 Hz).

Incubation of 2β-methoxyclovan-9α-ol (**2**) with *B. cinerea* yielded five compounds with a clovane skeleton (compounds **3**−**5**, **7**, and **9**). Ketone **3** and alcohols **4** and **9** did not persist for a long time in the culture medium (see Experimental Section). Biotransformation studies of 2β-methoxyclovan-9-one (**3**) and 2β-methoxyclovan-9β-ol (**4**) revealed their transformation to other compounds also obtained in the incubation of the H-9αalcohol **2**.

When clovan-2,9-dione (**6**) was incubated with *B. cinerea*, neither unchanged material nor any metabolite was isolated from the fermentation broth. Because clovan-2,9-dione (**6**) is a 1,5-diketone, it may undergo a series of *retro*-Michael reactions leading to fission of the ring system.

The antifungal properties of compounds **2**−**8** were determined against the growth of *B. cinerea* using the poisoned-food technique.[7] The commercial fungicide Euparen was used as a standard for comparison in this test. Several levels of inhibition were observed. Maximal inhibition was shown for compound **2**, and it was found to decrease in the sequence **4**>**3**>**7**>**5** (Figure 2).

These biotransformations are of interest for several reasons. First, the major metabolites of 2β-methoxyclovan-9α-ol (**2**) all show a much reduced biological activity when compared with the parent compound (see Scheme 3). Second, the inversion of configuration at C-9 only proceeded in the direction 9α-ol to 9β-ol, although the 9-ketone is a metabolite of the 9β-alcohol. The reduction at C-9 has followed the Prelog rule for the asymmetric reduction of ketones.[8] Third, the facile dealkylation of the 2β-methyl ether to generate the 2β,9α- and 2β,9β-diols would afford an easy route to the 2,9-dione, which does not persist in the medium. The existence of microbial detoxification pathways for these fungistatic agents suggests that they might not persist in the environment for a prolonged period. In light of this work, it is possible to represent the biodegradation of 2β-methoxyclovan-9α-ol (**1**) by *B. cinerea* in Scheme 3.

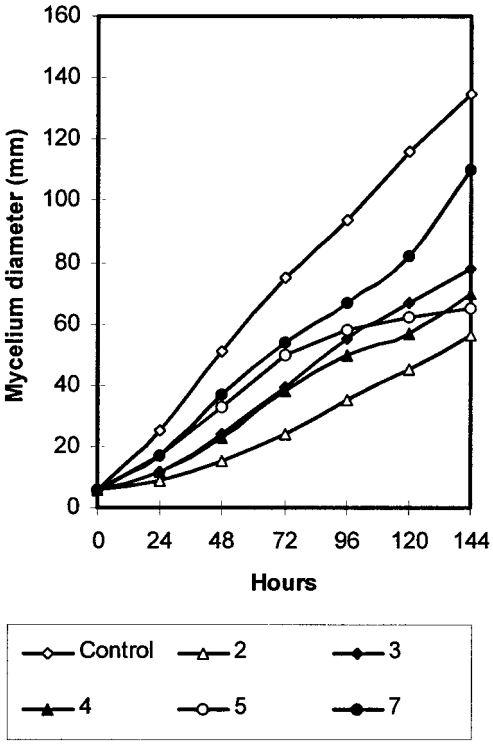
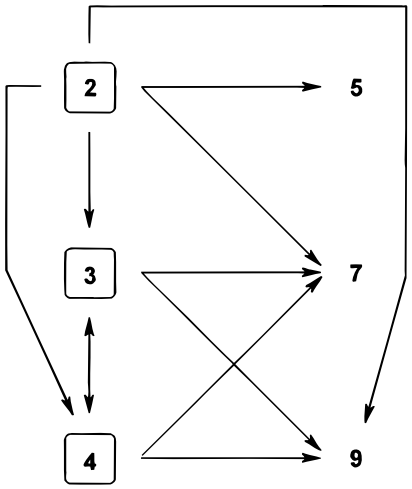


Figure 2. Comparison of fungal growth inhibition (*B. cinerea*) among compounds **2**−**5** and **7**.

Scheme 3. Metabolism of Clovanes by *B. cinerea*



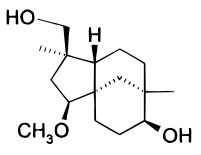
Experimental Section

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. ^1H and ^{13}C NMR measurements were obtained on Varian Gemini 200 and Varian Unity 400 NMR spectrometers with Me_4Si as internal reference. MS were recorded on 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. Si gel (Merck) was used for column chromatography. Purification by HPLC was accomplished using a Si gel column (Hibar 60, 7 m, 1 cm wide, 25 cm long).

Microorganism and Antifungal Assays. The culture of *B. 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/L of glucose−malt−peptone−agar, 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.

General Culture Conditions. *B. cinerea* (UCA 992) was grown on surface culture in Roux bottles at 25° C for 4 days on a Czapek−Dox medium (150 mL/flask) consisting of (per L of distilled H_2O) 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 sodium chloride, 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 sodium sulfate, the solvent was evaporated, and the residues were chromatographed on Si gel in a gradient mixture of petroleum ether−EtOAc of increasing polarity. The acidic fractions were methylated with CH_2N_2 prior to chromatography.

Biotransformation of 2*β*-Methoxyclovan-9α-ol (2) by *B. cinerea*. Compound **2** (60 mg) was distributed over 12 flasks of *B. cinerea* and the fermentation grown for a further 4, 9, and 16 days. Chromatography of the neutral fraction (4 days, 4 flasks) gave 2*β*-methoxyclovan-9α-ol (**2**)[3] (1 mg), 2*β*-methoxyclovan-9-one (**3**) (3 mg), 2*β*-methoxyclovan-9*β*-ol (**4**) (3 mg), clovan-2*β*,9α-diol (**5**)[9] (4 mg), clovan-2*β*,9*β*-diol (**7**) (8 mg), 2*β*-methoxyclovan-9*β*,13-diol (**9**) (12 mg), and dihydrobotrydial (**10**)[10] (8 mg). Chromatography of the neutral fraction (9 days, 4 flasks) gave 2*β*-methoxyclovan-9α-ol (**2**) (<1 mg), 2*β*-methoxyclovan-9-one (**3**) (<1 mg), 2*β*-methoxyclovan-9*β*-ol (**4**) (<1 mg), clovan-2*β*,9α-diol (**5**) (2 mg), clovan-2*β*,9*β*-diol (**7**) (4 mg), and dihydrobotrydial (**10**) (10 mg). Chromatography of the neutral fraction (16 days, 4 flasks) gave clovan-2*β*,9α-diol (**5**) (2 mg), clovan-2*β*,9*β*-diol (**7**) (2 mg), and dihydrobotrydial (**10**) (13 mg).



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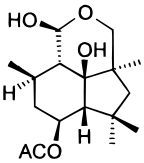

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Biotransformation of 2*β*-Methoxyclovan-9-one (3) by *B. cinerea*. Compound **3** (250 mg) was distributed over 35 flasks of *B. cinerea* and the fermentation grown for a further 10 days. Chromatography of the neutral fraction gave 2*β*-methoxyclovan-9*β*-ol (**4**) (10 mg), clovan-2*β*,9*β*-diol (**7**) (32 mg), 2*β*-methoxyclovan-9*β*,13-diol (**9**) (10 mg), and dihydrobotrydial (**10**) (7 mg).

Biotransformation of 2*β*-Methoxyclovan-9*β*-ol (4) by *B. cinerea*. Compound **4** (250 mg) was distributed over 35 flasks and the fermentation grown for a further 10 days. Chromatography of the neutral fraction gave 2*β*-methoxyclovan-9-one (**3**) (8 mg), clovan-2*β*,9*β*-diol (**7**) (19 mg), 2*β*-methoxyclovan-9*β*,13-diol (**9**) (13 mg), and dihydrobotrydial (**10**) (7 mg).

Biotransformation of Clovan-2,9-dione (6) by *B. cinerea*. Incubation of compound **6** (150 mg) distributed over 21 flasks for 4 days gave dihydrobotrydial (**10**) (120 mg), but neither the starting material nor any clovane metabolites was detected.

2*β*-Methoxyclovan-9-one (3): obtained as an oil; $[\alpha]^{25}_D$ −63.9° (*c* 0.013, $CHCl_3$); IR (film) ν_{max} 2938, 2866, 2823, 1711, 1509, 1461, 1361, 1367, 1151, 1113, 1193, 992, 922, 836, cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.95 (3H, s, H-13α), 1.00 (3H, s, H-15), 1.06 (3H, s, H-14*β*), 1.4−1.6 (6H, H-6, H-6′, H-7, H-7′, H-12, H-12′), 1.57 (1H, dd, $J_{3\beta-2\alpha} = J_{3\beta-3\alpha} = 12.8$ Hz, H-3*β*), 1.79 (1H, dd, $J_{3\alpha-3\beta} = 12.8$ Hz, $J_{3\alpha-2\alpha} = 5.5$ Hz, H-3α), 1.86 (1H, ddd, $J_{11'-11} = 13.6$ Hz, $J_{11'-10'} = 12.4$ Hz, $J_{11'-10} = 6.8$ Hz, H-11′), 2.33 (1H, ddd, $J_{10-10'} = 16.4$ Hz, $J_{10-11} = 2.4$ Hz, $J_{10-11'} = 6.8$ Hz, H-10), 2.61 (1H, ddd, $J_{10-10'} = 16.4$ Hz, $J_{10'-11} = 12.4$ Hz, $J_{10'-11} = 7.8$ Hz, H-10′), 3.29 (3H, s, OMe), 3.45 (1H, dd, $J_{2\alpha-3\alpha} = 5.5$ Hz, $J_{2\alpha-3\beta} = 12.8$ Hz, H-2α); ^{13}C NMR ($CDCl_3$, 50 MHz), see Table 2; EIMS *m/z* (70 eV) 251 (4) [$M^+ + 1$], 250 (23) [M^+], 235 (15) [$M^+ - 15$], 218 (12) [$M^+ - MeOH$], 203 (13) [$M^+ - 15 - 28$], 194 (100) [$M^+ - CO - C_2H_4$], 163 (39), 161 (25), 149 (33), 147 (25), 135 (36), 121 (90), 107 (55), 105 (58), 99 (61), 98 (51); HREIMS *m/z* 250.1932 ($C_{16}H_{26}O_2$ requires 250.1920).

2*β*-Methoxyclovan-9*β*-ol (4): obtained as an oil; $[\alpha]^{25}_D$ +23.9° (*c* 0.0108, $CHCl_3$); IR (film) ν_{max} 3420, 2938, 1466, 1367, 1280, 1196, 1101, 1056, 1024, 966 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.84 (3H, s, H-13α), 0.98 (3H, s, H-15), 1.02 (3H, s, H-14*β*), 1.08 (1H, d, $J_{12\beta-12\alpha} = 13.2$ Hz, H-12*β*), 1.12−1.32 (4H, H-6, H-6′, H-7, H-11), 1.28 (1H, d, $J_{12\beta-12\alpha} = 13.2$ Hz, H-12α), 1.29 (1H, m, H-5*β*), 1.32−1.52 (3H, H-7′, H-11, H-10), 1.42 (1H, dd, $J_{3\beta-2\alpha} = J_{3\beta-3\alpha} = 11.1$ Hz, H-3*β*), 1.70 (1H, dd, $J_{3\alpha-2\alpha} = 5.6$ Hz, $J_{3\beta-3\alpha} = 11.1$ Hz, H-3α), 1.71 (1H, m, H-10′), 3.18 (1H, dd, $J_{9\alpha-10\beta} = 10.4$ Hz, $J_{9\alpha-10\alpha} = 5.0$ Hz, H-9α), 3.32 (1H, dd, $J_{2\alpha-3\alpha} = 5.6$ Hz, $J_{2\alpha-3\beta} = 11.1$ Hz, H-2α), 3.31 [3H, s, −OMe(H-1′)]; ^{13}C NMR ($CDCl_3$, 50 MHz) see Table 2; EIMS *m/z* (70 eV) 252 (0.3) [M^+], 237 (6) [$M^+ - 15$], 234 (10) [$M^+ - H_2O$], 220 (5) [$M^+ - MeOH$], 219 (5) [$M^+ - MeOH - H^+$], 202 (19) [$M^+ - H_2O - MeOH$], 187 (10) [$M^+ - H_2O - MeOH$-15], 178 (8), 161 (12), 150 (12), 135 (19), 121 (7), 107 (17), 105 (26), 99 (100), 91 (21), 85 (17), 79 (18); HREIMS *m/z* 252.2089 ($C_{16}H_{28}O_2$ requires 252.2077).

Clovan-2*β*,9*β*-diol (7): obtained as colorless crystals; mp 172−174 °C (lit.[14] 174−175 °C); $[\alpha]^{25}_D$ +15.3° (*c* 0.0028, $CHCl_3$); IR (film) ν_{max} 3329, 2929, 1463, 1461, 1361, 1269, 1211, 1166, 1120, 1061, 1016, 681 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.85 (3H, s, H-13α), 0.99 (3H, s, H-15), 1.03 (1H, d, $J_{12-12'} = 12.8$ Hz, H-12), 1.05 (3H, s, H-14*β*), 1.23 (1H, d, $J_{12-12'} = 12.8$ Hz, H-12′), 1.15−1.25 (2H, H-6, H-7), 1.28−1.40 (3H, H-11, H-11′, H-5*β*), 1.40−1.50 (2H, H-6′, H-7′), 1.45 (1H, dd, $J_{3\beta-2\alpha} = J_{3\beta-3\alpha} = 12.0$ Hz, H-3*β*), 1.53 (1H, m, H-10*β*), 1.71 (1H, dd, $J_{3\alpha-2\alpha} = 5.6$ Hz, $J_{3\beta-3\alpha} = 12.0$ Hz, H-3α), 1.76 (1H, dddd, $J_{10-10'} = 12.8$ Hz, $J_{10'-9\alpha} = 4.8$ Hz, $J = 3.3, 3.3$ Hz, H-10α), 3.20 (1H, dd, $J_{9\alpha-10} = 10.8$ Hz, $J_{9\alpha-10'} = 4.8$ Hz, H-9α), 3.80 (1H, dd, $J_{2\alpha-3\alpha} = 5.6$ Hz, $J_{2\alpha-3\beta} = 12.0$ Hz, H-2α); ^{13}C NMR ($CDCl_3$, 50 MHz) see Table 2; EIMS *m/z* (70 eV) 238 (2.3) [M^+], 220 (58) [$M^+ - H_2O$], 205 (35) [$M^+ - H_2O - 15$], 202 (42) [$M^+ - 2H_2O$], 187 (24) [$M^+ - 2H_2O - 15$], 182 (60), 178 (42), 164 (55), 163 (65), 150 (28), 149 (42), 135 (61), 133 (35), 123 (47), 121 (43), 109 (33), 108 (53), 105 (68), 95 (64), 93 (68), 85 (55), 81 (60), 55 (73); HREIMS *m/z* 238.1921 ($C_{16}H_{28}O_2$ requires 238.1905).

2*β*-Methoxyclovan-9*β*,13-diol (9): obtained as colorless needles; mp 157−158 °C; $[\alpha]^{25}_D$ + 30.0° (*c* 0.0098, $CHCl_3$); IR (film) ν_{max} 3279, 1464, 1377, 1192, 1114, 1054, 723 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.85 (3H, s, H-13α), 1.04 (3H, s, H-15), 1.15 (1H, dd, $J = 3.0, 12.7$ Hz, H-5*β*), 3.33 (3H, s, OMe), 3.35 (1H, m, H-2α), 3.46 (1H, d, $J_{14-14'} = 10.4$ Hz, H-14), 3.53 (1H, d, $J_{14-14'} = 10.4$ Hz, H-14′), 3.57 (1H, dd, $J = 10.9, 5.1$ Hz, H-9α); ^1H NMR (pyridine-d_5, 200 MHz) δ 0.82 (3H, s, H-13α), 1.02 (3H, s, H-15), 3.29 (3H, s, O*Me*), 3.40 (1H, dd, $J_{2\alpha-3\alpha} = 5.6$ Hz, $J_{2\alpha-3\beta} = 10.0$ Hz H-2α), 3.86 (1H, d, $J_{14-14'} = 10.0$ Hz H-14), 3.98 (d, 1H, $J_{14'-14} = 10.0$ Hz H-14′), 3.94−4.00 (1H, m, obscured by doublet of H-14′, H-9α); ^{13}C NMR ($CDCl_3$, 50 MHz) see Table 2; EIMS *m/z* (70 eV) 250 (19) [$M^+ - H_2O$], 235 (14), 218 (55) [$M^+ - H_2O - MeOH$], 200 (40) [$M^+ - H_2O - MeOH - H_2O$], 187 (37), 174 (52), 161 (81), 145 (36),

131 (45), 119 (40), 107 (45), 99 (100), 91 (60), 69 (54), 55 (46), 41 (74), 28 (50). Anal. C 71.61%, H 10.7%, calcd for $C_{16}H_{28}O_3$, C 71.60%, H 10.5%.

Oxidation of 2*β*-Methoxyclovan-9α-ol (4) with Jones' Reagent. A solution of compound **2** (60 mg)[3] in Me_2CO (10 mL) was tritiated with a solution of Jones' reagent[5] until the red color of the reagent persisted. TLC control of the mixture confirmed that starting material had reacted, and solvent was evaporated under reduced pressure. The resulting gum was redissolved in EtOAc, washed with a saturated solution of $NaHCO_3$ in H_2O, washed with brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a crude reaction product that was further purified by column chromatography on Si gel, with increasing gradients of EtOAc in petroleum ether, to yield 2*β*-methoxiclovan-9-one (**3**) (57 mg, 97%).

$LiALH_4$ Reduction of 2*β*-Methoxyclovan-9-one (3). $LiAlH_4$ (30 mg) was added to a stirred solution of compound **3** (46 mg) in dry dimethyl ether (10 mL), under an inert atmosphere (N_2). When the ketone was consumed (24 h) excess $LiAlH_4$ was destroyed by careful addition of H_2O. The resulting suspension was extracted twice with EtOAc, and the organic phase was dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a crude reaction product that was further purified by column chromatography on Si gel, with increasing gradients of EtOAc in petroleum ether, to yield 2*β*-methoxyclovan-9*β*-ol (**4**) (30 mg, 65%).

Oxidation of Compound 7 with Pyridinium Chlorochromate (PCC). PCC (262 mg) was added to a stirred solution of **5** (287 mg)[6d] in CH_2Cl_2 (30 mL). When TLC control of the mixture confirmed that the starting material had reacted (5 h), the solvent was evaporated under reduced pressure. The resulting gum was redissolved in Et_2O, washed with a saturated solution of $NaHCO_3$ in H_2O, washed with brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a crude reaction product that was further purified by column chromatography on Si gel, with increasing gradients of EtOAc in petroleum ether, to yield clovan-2,9-dione (**6**) (244 mg, 86%).

Clovan-2,9-dione (6): obtained as colorless crystals; mp 47−48 °C; $[\alpha]^{25}_D$ −97.0° (*c* 0.01325, $CHCl_3$); IR (film) ν_{max} 2953, 2933, 2870, 1735, 1710, 1458, 1420, 1372, 1344, 1311, 1195, 1281, 1220, 1139, 1105, 886, 836 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.02 (3H, s, H-15), 1.07 (3H, s, H-13α), 1.13 (3H, s, H-14*β*), 1.45 (1H, ddd, $J_{7-6'} = J_{7-6} = 7.4$ Hz, $J_{7-7'} = 14.5$ Hz, H-7), 1.54−1.66 (5H, H-12, H-12′, H-6, H-7′, H-11), 1.73 (1H, m, H-6′), 1.94 (1H, dd, $J_{5\beta-6} = 6.8$ Hz, $J_{5\beta-6'} = 6.8$ Hz, H-5*β*), 2.14 (1H, ddd, $J_{11'-10} = 7.5$ Hz, $J_{11'-10'} = 11.3$ Hz, $J_{11'-11} = 13.1$ Hz, H-11′), 2.19 (1H, d, $J_{3-3'} = 17.5$ Hz, H-3), 2.36 (1H, d, $J_{3-3'} = 17.5$ Hz, H-3′), 2.42−2.58 (2H, H-10, H-10′); ^{13}C NMR ($CDCl_3$, 50 MHz) see Table 2; EIMS *m*/*z* (70 eV) 234 (6) [M^+], 178 (100) [M^+-56], 165 (17), 150 (39), 135 (22), 107 (26), 93 (39), 91 (31), 79 (33), 77 (23); HREIMS *m*/*z* 234.1620 ($C_{15}H_{22}O_2$ requires 252.2082).

$NaBH_4$ Reduction of Clovan-2,9-dione (6). Sodium borohydride (21 mg) was added to a stirred solution of compound **6** (122 mg) in MeOH (20 mL). Once the ketone was consumed (5 h), excess $NaBH_4$ was destroyed by the careful addition of H_2O. The resulting suspension was extracted twice with EtOAc, and the organic phase was dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a crude reaction product that was further purified by column chromatography on Si gel, with increasing gradients of EtOAc in petroleum ether, to yield clovan-2α,9*β*-diol (**8**) (102 mg, 82%), clovan-2*β*,9*β*-diol (**7**) (6 mg, 5%), and clovan-2*β*,9α-diol (**5**) (4 mg, 3%).

Clovan-2α,9*β*-diol (8): obtained as colorless crystals; mp 177.5−178.5 °C; $[\alpha]^{25}_D$ −19.7° (*c* 0.0163, $CHCl_3$); IR (film) ν_{max} 3355, 2953, 2864, 1458, 1350, 1321, 1273, 1244, 1209, 1130, 1059, 1025, 1004, 926, 859, 759, 674, 633 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.81 (1H, d, $J_{12\alpha-12\beta} = 12.8$ Hz, H-12α), 0.97 (6H, s, H-13, H-14), 1.01 (3H, s, H-15), 1.15−1.40 (2H, H-6, H-7), 1.25 (1H, m, H-11), 1.31 (1H, dd, $J_{12\alpha-12\beta} = 12.8$ Hz, $J_{12\beta-11\beta} = 3$ Hz, H-12*β*), 1.48−1.65 (5H, H-10, H-11′, H-5*β*, H-6′, H-7′), 1.54 (1H, dd, $J_{3-3'} = J_{3-2\beta} = 12.0$ Hz, H-3), 1.70 (1H, dd, $J_{3-3'} = 12.0$ Hz, $J_{3'-2\beta} = 6.4$ Hz, H-3′), 1.77 (1H, m, H-10′), 3.24 (1H, dd, $J = 5.6$, 10.8 Hz, H-9α), 3.86 (1H, dd, $J_{2\beta-3'} = 5.6$ Hz, $J_{2\beta-3'} = 12.0$ Hz, H-2*β*); ^1H NMR (CD_3OD, 400 MHz) δ 0.78 (1H, d, $J_{12\alpha-12\beta} = 12.8$ Hz, H-12α), 0.94 (6H, s, H-13, H-14), 0.95 (3H, s, H-15), 1.08−1.30 (3H, H-6, H-7, H-11), 1.20 (1H, m, H-5*β*), 1.32 (1H, dd, $J_{12\alpha-12\beta} = 12.8$ Hz, $J_{12\beta-11\beta} = 3$ Hz, H-12*β*), 1.46−1.55 (3H, H-11′, H-6′, H-7′), 1.50−1.55 (2H, H-3, H-3′), 1.55−1.65 (2H, H-10, H-10′), 3.13 (1H, dd, $J = 5.6$, 10.8 Hz, H-9α), 3.76 (1H, dd, $J = 6.4$, 10.0 Hz, H-2*β*); ^{13}C NMR ($CDCl_3$, CD_3OD 50 MHz) see Table 2; EIMS *m*/*z* (70 eV) 238 (63) [M^+], 223 (13) [$M^+ - 15$], 220 (75) [$M^+ - H_2O$], 205 (35) [$M^+ - H_2O - 15$], 202 (38) [M^+-$2H_2O$], 187 (24) [M^+-$2H_2O$-15], 182 (44), 179 (79), 163 (100), 161 (71), 151 (50), 135 (55), 123 (62), 107 (65), 105 (74), 95 (59), 93 (56), 81 (43), 79 (37), 69 (33), 67 (27); HREIMS *m*/*z* 238.1921 ($C_{16}H_{28}O_2$ requires 238.1930).

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