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Aromadendrane transformations by *Curvularia lunata* ATCC 12017

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Dedicated to Professor Sir John Cornforth, University of Sussex as he enters his 85th year.

Abstract

The naturally occurring sesquiterpene squamulosone (**1**), isolated from *Hyptis verticillata* (Labiatae), was synthetically reduced to five analogues that were identified as (1*S*,10*S*)-9α-hydroxy-*allo*-aromadendrane (**2**), (1*R*,10*R*)-9β-hydroxyaromadendrane (**3**), (1*S*,10*S*)-*allo*-aromadendran-9-one (**4**), (1*R*,10*R*)-aromadendran-9-one (**5**) and aromadendra-1,9-diene (**6**). Each congener was incubated with the fungus *Curvularia lunata* ATCC 12017 in two different growth media. All the substrates except the deoxy compound **6** underwent a simple redox reaction. Ketone **5** additionally experienced remote hydroxylation while analogue **6**, possessing a conjugated diene system, was most extensively metabolised. The substrates and products presented here, but one, are all novel.

Keywords: *Curvularia lunata*; Aromadendrane; Sesquiterpene; Biotransformation; Hydroxylation

1. Introduction

Curvularia lunata is a deuteromycete widely known for its 11β-hydroxylation of steroids (Chen and Wey, 1990) and chiefly its employment in the quantitative conversion of cortexolone to the anti-inflammatory agent cortisol (Holland, 1992). The fungus has also been studied in the metabolism of a number of terpenes (Azerad, 2000; Ismaili-Alaoui et al., 1992; Garcia-Granados et al., 1991; de Lima et al., 1999; Aranda et al., 1991) with the most recent investigations focusing on the bioconversion reactions of the organism under different growth conditions (Collins et al., 2001; Collins and Reese, 2001).

In a continuing effort to generate terpenes with potentially enhanced biological activity (Collins et al., 2001) synthetically derived compounds **2**, **3**, **4**, **5** and **6** were incubated with *C. lunata* ATCC 12017 (synonyms: IMI 61535, CBS 215.54, NRRL 2380) in two different growth media: potato dextrose broth (PDB) (Chen and Wey, 1990) and a beef extract medium (BEM) (Garcia-Granados et al., 1991). Studies in our group have indicated that the nature of the docking group (carbonyl, hydroxyl, etc.) in the substrate will usually modify the range and yield of the products of transformation (Buchanan and Reese, 2001). The novel substrates (**2**–**6**) were obtained by reduction of the naturally occurring sesquiterpene squamulosone (**1**), an insecticidal terpene isolated in large quantities from the folk medicinal plant *Hyptis verticillata*. Compound **1** was previously fed to *C. lunata* (Collins et al., 2001).

2. Results and discussion

Squamulosone (**1**) was chemically reduced to five analogues, namely: (1*S*,10*S*)-9α-hydroxy-*allo*-aromadendrane (**2**), (1*R*,10*R*)-9β-hydroxyaromadendrane (**3**), (1*S*,10*S*)-*allo*-aromadendran-9-one (**4**), (1*R*,10*R*)-aromadendran-9-one (**5**) and aromadendra-1,9-diene (**6**). Alcohols **2** and **3** were both derived from a low temperature hydride reduction of the inseparable ketone mixture that had been obtained when squamulosone (**1**) was hydrogenated. The alcohols were then re-oxidised to ketones **4** and **5** respectively.

Data from the HR(EI)MS of **2** suggested a molecular formula of $C_{15}H_{26}O$ ($[M]^+ = 222.1982$) that corresponded to the reduction of the α,β-unsaturated system

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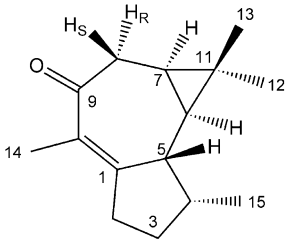

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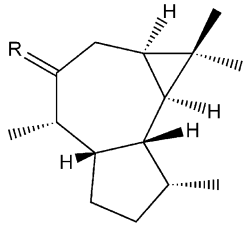
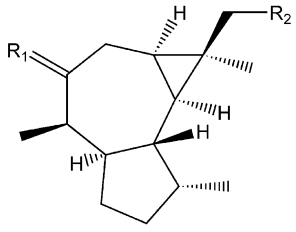


2: R = αOH, βH
4: R = O
7: R = βOH, αH



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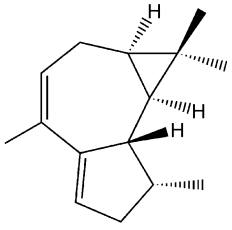
5: R_1 = O, R_2 = H
8: R_1 = O, R_2 = OH



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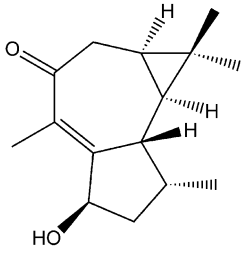

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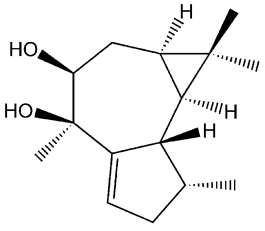

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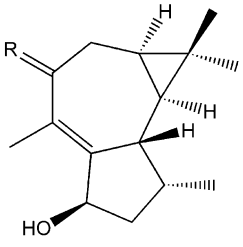
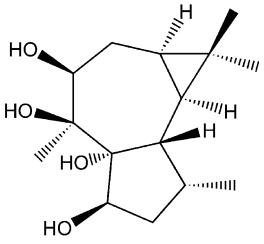


11: R = βOH, αH
12: R = αOH, βH



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of squamulosone to form a saturated alcohol. The consequent stereochemical questions regarding the hydroxyl, methyl, and ring junction were addressed using a T-ROESY NMR experiment. Important was an observed ROE between H-1 (δ 2.16) and H-4 (δ 1.90) that pointed to the presence of a *cis* ring junction. This effectively meant that hydrogenation had occurred from the upper face of the molecule. This was further supported by enhancements of H-14 (δ 1.10) and H-10 (δ 1.76) upon the respective irradiations of H_R-8 (δ 1.27) and H-9 (δ 3.80). Other couplings between H-9 and H-5 (δ 1.60) and also H_S-8 (δ 2.26) indicated an α-stereochemistry for the hydroxyl substituent. Derivative **3**, having a suggested molecular formula of $C_{15}H_{26}O$ ($[M]^+ = 222.1983$), was also a saturated alcohol. In this case hydrogenation of **1** had occurred from the lower face of the molecule resulting in a *trans* ring junction. This proposition was supported by the T-ROESY data that correlated H-1 (δ 1.94) to both the H-6 (δ 0.24) and H-7 (δ 0.52) protons. The C-9 hydroxyl group was assigned a β-stereochemistry because of the observed ROE between H-9 (δ 3.87) and H_R-8 (δ 2.22) and also H-10 (δ 1.93). The coupling constants of H-9 (*dt*, $J = 2.4$, 8.4 Hz) were also consistent with this assignment. Both **2** and **3** were oxidised to their corresponding ketones (**4** and **5**). The existence of a carbonyl in each molecule was established by the presence of a typical absorption band (ν_{max} 1709 cm^{-1} for **4** and 1703 cm^{-1} for **5**) in the IR spectrum. HR(EI)MS data ($[M]^+ = 220.1827$ and 220.1829 for **4** and **5**, respectively) that indicated molecular formulae of $C_{15}H_{24}O$ were also in line with expectations. HR(EI)MS data for **6** ($[M]^+ = 202.1721$) suggested that the compound was a hydrocarbon ($C_{15}H_{26}$). The five degrees of unsaturation were accounted for by the presence of two olefinic bonds within the tricyclic system. A UV absorption band at λ_{max} 242 nm further indicated that this compound was a heteroannular diene.

The incubation of alcohol **2** in both fermentation media resulted in the isolation of ketone **4** from the broth. An additional metabolite, (1*S*,10*S*)-9β-hydroxy-*allo*-aromadendrane (**7**), was obtained with the use of BEM. HR(EI)MS data for **7** pointed to a molecular formula of $C_{15}H_{26}O$ ($[M]^+ = 222.1985$). The signal at δ_C 73.5 was ascribed to a hydroxyl group (IR ν_{max} 3201 cm^{-1}) that was attached to C-9 based on HMQC, HMBC and ^1H–^1H COSY data. The β-stereochemistry of the hydroxyl function was deduced from T-ROE enhancements observed between H_R-8 (δ_H 2.03) and both H-7 (δ 0.61) and H-9 (δ 3.48). This meant that **7** was the C-9 epimer of **2** with its formation necessarily involving **4** as an intermediate.

Substrate **3** was poorly metabolised by *C. lunata* in both growth media to give ketone **5** as the sole product. The bioreaction on **4** was limited to a simple reduction that yielded **7** in both PDB and BEM. In addition, metabolite **2** was obtained from **4** when the fungus was grown in PDB.

Incubation of **5** resulted in the isolation of **3** from both fermentations. Substrate **5** was also converted to (1*R*,10*R*)-13-hydroxyaromadendr-9-one (**8**) when BEM was used. The HR(EI)MS data of **8** suggested a molecular formula of $C_{15}H_{24}O_2$ ($[M]^+ = 236.1776$) and as such highlighted the presence of an additional hydroxyl

group. The oxygen-bearing carbon (δ 72.8), observed as a methylene in DEPT NMR experiments, was assigned to C-13 based on a comparison of the carbon resonances of **8** with those of **5**.

The metabolism of the deoxy compound **6** with *C. lunata* produced five metabolites in both media that were identified as 2β-hydroxyaromadendr-1(10)-en-9-one (**9**), 9β,10β-dihydroxyaromadendr-1-ene (**10**), 2β,9β-dihydroxyaromadendr-1(10)-ene (**11**), 2β,9α-dihydroxyaromadendr-1(10)-ene (**12**), and (1*S*,10*S*)-1α,2β,9β, 10β-tetrahydroxyaromadendrane (**13**). The known compound **9** was identified by comparison of its spectral data with those in the literature (Collins et al., 2001). Metabolite **10** had a molecular formula of $C_{15}H_{24}O_2$ ($[M]^+ = 236.1776$) and its two oxygen functions, borne by a methinic (δ 76.4) and a non-protonated (δ 73.5) carbon, were exposed as hydroxyls in the IR spectrum (ν_{max} 3392 cm^{-1}). The retention of the trisubstituted double bond within the cyclopentane ring was also evident (δ_H 5.70, *dd*) and the chemical shifts of C-7, C-8 and C-14 were consistent with oxygen insertion at C-9 and C-10. The proposed structure was confirmed by T-ROESY data that revealed unambiguous correlations between H-9 (δ 3.39), H-7 (δ 0.50) and H-14 (δ 1.51). Congener **11**, like **10**, had a molecular formula of $C_{15}H_{24}O_2$ ($[M]^+ = 236.1776$). The two oxygen-bearing methines (δ_C 74.0 and 75.1) present, however, together with two non-protonated olefinics (δ_C 151.8 and 126.8) strongly suggested that oxygen insertion was accompanied by migration of the double bond to form the more stable tetrasubstituted alkene. HMBC data positioned the hydroxyls at C-2 (74.0 ppm) and C-9 (75.1 ppm) and both were assigned β-stereochemistries based on T-ROESY couplings. The formation of **10** and **11** possibly involves the initial epoxidation of the C-9,10 double bond from the β-face. The epoxide then opens in the presence of water, via an S_N2' type mechanism, to give **11** which itself could undergo an S_N2' reaction to form **10**. 2β,9α-Dihydroxyaromadendr-1(10)-ene (**12**) was also isolated. While the ^{13}C and ^1H NMR data were similar to those of **11** it was noted that there was a difference in the multiplicity and coupling constants for the H-9 signal (δ 4.30, *t*, $J = 2.5$ Hz). The formation of **9** and **12** could be rationalised using **11** as an intermediate. Oxidation of the latter would give **9** which could then be reduced to **12**. The most polar metabolite (**13**) had four oxygen-bearing carbons in the ^{13}C NMR spectrum that were inferred from IR data to be hydroxyls. The lack of any resonance in the olefinic region suggested that the four oxygen functions were positioned at C-1 (δ 69.4), C-2 (δ 61.9), C-9 (δ 65.5) and C-10 (δ 58.3). The β-stereochemistry of the C-9 and C-10 were determined as in **10** and that of C-2 followed from observed T-ROE couplings between H-2 (δ 3.50), H_S-3 (δ 1.37) and H-6 (δ 0.16). It is proposed that epoxidation of **11** followed by ring opening of the resulting oxirane by water would yield the *trans*-diol **13**.

In summary hydrogenation of squamulosone (**1**) followed by low temperature hydride reduction produced two alcohols, **2** and **3**, which were then oxidised to the corresponding ketones **4** and **5**. One deoxy product (**6**) was obtained from **1** by hydride reduction at room temperature. These novel substrates, **2**–**6**, were incubated with *C. lunata* in two growth media, however, the reactions were generally consistent across both media. The alcohols and ketone **4** experienced only simple redox reactions while **5** was the only substrate to be remotely hydroxylated and this was at the C-13 methyl. Analogue **6** produced five metabolites as a result of reactions at the activated carbons of the double bonds, and all but one of these are new.

3. Experimental

^1H and ^{13}C NMR spectra were generated in deuterated chloroform at 200 and 50 MHz respectively using a Brüker AC200 instrument. 2D NMR data were also generated in chloroform at 500 and 125 MHz respectively using a Varian Unity 500 NMR spectrometer. Tetramethylsilane (TMS) was the internal standard. ^{13}C NMR assignments for the aromadendranes are listed in Table 1. Hydrogenation was done using a PARR 3911 hydrogenator. Melting points were determined on a Thomas-Hoover capillary melting point apparatus. Infrared data were acquired on a Perkin Elmer FTIR Paragon 1000 instrument using KBr disks. UV spectra were determined on a Hewlett Packard HP 8452A spectrophotometer. Optical rotations were acquired on a Perkin Elmer 241 polarimeter. High-resolution electron impact mass spectrometry (HREIMS) was performed on a VG 70–250S instrument at an ionising voltage of 70 eV. Column chromatography was performed with Kieselgel silica (40–63 μm). Compounds on TLC plates were visualised by the use of the ammonium molybdate/sulfuric acid spray reagent followed by heating at 120 °C. *C. lunata* ATCC 12017 was obtained from the American Type Culture Collection, Rockville, MD, USA. Squamulosone (**1**) was isolated from the plant *H. verticillata* (Collins et al., 2001). Petrol refers to the petroleum fraction boiling between 60 and 80 °C.

3.1. Feeding protocol

The fungus was maintained on potato dextrose agar (PDA) slants. PDB was comprised of potato dextrose broth (24 g l^{-1}) (Chen and Wey, 1990). BEM was composed of peptone (1 g l^{-1}), yeast extract (1 g l^{-1}), beef extract (1 g l^{-1}) and glucose (5 g l^{-1}) (Garcia-Granados et al., 1991). One 14 day old slant was used to inoculate four 500 ml conical flasks each containing 125 ml of liquid medium. The flasks were shaken at 200 rpm at 27 °C. An EtOAc solution (2 ml) containing 10% of the

Table 1
^{13}C NMR resonances for aromadendranes **1–13**

Carbon	1	2	3	4	5	6	7	8	9	10	11	12	13
1	166.4	50.0	44.4	48.9	49.7	148.4	49.2	49.7	163.2	151.8	146.3	145.9	69.4
2	34.1	25.6	30.84	24.0	30.48	123.3	23.5	30.4	74.2	126.8	74.0	74.4	61.9
3	32.2	29.4	30.87	29.0	30.48	39.0	29.1	30.5	41.5	37.5	41.1	42.0	33.3
4	37.2	37.9	38.0	37.8	37.6	34.5	38.1	37.8	33.8	42.8	39.4	39.7	38.4
5	45.2	43.0	41.4	43.1	42.3	47.0	42.8	41.3	42.5	38.1	33.6	33.4	29.9
6	31.3	23.2	22.8	23.8	23.5	28.6	22.6	20.0	28.2	24.8	30.0	30.2	22.7
7	22.8	20.6	21.7	22.4	19.8	26.6	24.2	16.6	19.2	23.0	21.2	23.0	23.5
8	41.9	30.8	31.4	40.1	38.9	24.1	35.5	38.3	41.3	29.7	31.7	29.9	25.5
9	201.0	75.7	75.5	211.8	215.5	126.7	73.5	214.8	201.2	76.4	75.1	73.9	65.5
10	130.3	41.4	40.5	49.1	48.3	132.8	44.4	48.3	134.1	73.5	133.1	132.8	58.3
11	25.7	17.9	18.1	20.7	19.9	20.3	18.8	26.5	31.6	19.6	21.6	18.1	19.1
12	16.0	15.86	15.4	15.6	15.6	15.7	16.0	11.4	11.6	15.7	15.8	15.9	15.3
13	28.2	28.7	28.7	28.4	28.6	28.4	28.7	72.8	71.9	28.4	28.3	28.2	28.2
14	14.9	16.3	16.1	14.3	13.9	22.2	18.3	13.9	14.7	25.2	18.6	18.8	29.8
15	15.4	15.86	17.5	16.2	16.1	16.2	16.2	16.0	15.2	15.0	15.2	15.2	14.3

total mass of the substrate (200 mg) was fed 24 h after inoculation. The remaining 20, 30 and 40% portions of the substrate, in EtOAc, were fed at 36, 48 and 60 h respectively after inoculation. The fermentation was allowed to proceed for a further 10 days. The pH was measured (usually ~6.5) and the mycelium was filtered from the broth. Broth extraction utilised EtOAc (3×500 ml). The mycelium was homogenised in EtOAc. The extracts were dried with sodium sulfate, concentrated in vacuo, and analysed by thin layer chromatography for the presence of biotransformed compounds. The broth and mycelial extracts of each fermentation were then combined and subjected to column chromatography using increasing concentrations of EtOAc in petrol.

3.2. (1S,10S)-9α-Hydroxy-allo-aromadendrane (2) and (1R,10R)-9β-hydroxyaromadendrane (3)

Squamulosone (**1**) (3.2 g, 14.7 mmol) was dissolved in EtOAc (250 ml), Pd/C (5%, 200 mg) was added and hydrogen gas (15 psi) was applied. The flask was shaken for 8 h and the solution was filtered through a no. 3 sintered glass crucible. The solvent was then removed in vacuo. A mixture of two saturated ketones was evident from NMR analysis. This mixture was dissolved in THF (100 ml) and cooled to −70 °C (acetone–dry ice). Lithium aluminium hydride (0.541 g, 14.6 mmol) was added over 10 min and the temperature of the flask was maintained for 30 min and then allowed to attain 0 °C. The addition of water preceded extraction with EtOAc (3×300 ml). The extract was concentrated in vacuo and chromatographed using 2% EtOAc in petrol to yield (1*S*,10*S*)-9α-hydroxy-*allo*-aromadendrane (**2**) (1.566 g, 7.1 mmol). Oil; [α]: −71.4° (*c* = 23.2, $CHCl_3$); IR ν_{max} cm^{-1}: 3430, 2953, 1455; EIMS *m*/*z* (rel. int.): 222.1983 (23) $[M]^+$ (calc. 222.1984 for $C_{15}H_{26}O$), 204.1879 (40), 161.1332 (66), 135.1175 (23), 123.1166 (25), 122.1093 (41), 109.1016 (31), 95.0862 (100); ^1H NMR: δ 0.31 (1H, *t*, *J* = 9.3 Hz, H-6), 0.71 (1H, *ddd*, *J* = 3.0, 9.3, 10.8 Hz, H-7), 0.93 (3H, *d*, *J* = 6.6 Hz, H-15), 1.00 (3H, *s*, H-12), 1.08 (3H, *s*, H-13), 1.10 (3H, *d*, *J* = 6.6 Hz, H-14), 1.13 (1H, *m*, $^w/_2$ = 15.0 Hz, H-3), 1.27 (1H, *m*, $^w/_2$ = 13.9 Hz, H-8), 1.60 (1H, *m*, $^w/_2$ = 11.0 Hz, H-5), 1.69 (1H, *m*, $^w/_2$ = 14.3 Hz, H-3), 1.72 (1H, *m*, $^w/_2$ = 15.0 Hz, H-2), 1.74 (1H, *m*, $^w/_2$ = 13.3 Hz, H-2), 1.76 (1H, *m*, $^w/_2$ = 13.6 Hz, H-10), 1.90 (1H, *m*, $^w/_2$ = 23.1 Hz, H-4), 2.16 (1H, *m*, $^w/_2$ = 7.0 Hz, H-1), 2.26 (1H, *m*, $^w/_2$ = 7.0 Hz, H-8), 3.80 (1H, *dd*, *J* = 1.3, 7.0 Hz, H-9).

Further elution with 4% EtOAc in petrol led to the isolation of (1*R*,10*R*)-9β-hydroxyaromadendrane (**3**) (0.898 g, 4.0 mmol). Needles from acetone, mp 63.5–65 °C, [α]: +28.9° (*c* = 50.4, $CHCl_3$); IR ν_{max} cm^{-1}: 3364, 2953, 1456; EIMS *m*/*z* (rel. int.): 222.1982 (17) $[M]^+$ (calc. 222.1984 for $C_{15}H_{26}O$), 204.1879 (29), 189.1643 (22), 161.1330 (61), 135.1174 (29), 124.1251 (73), 123.1171 (28), 109.1017 (28), 95.0863 (100); ^1H NMR: δ 0.24 (1H, *dd*, *J* = 9.0, 11.6 Hz, H-6), 0.52 (1H, *ddd*, *J* = 5.0, 9.0, 12.8 Hz, H-7), 0.93 (3H, *d*, *J* = 6.8 Hz, H-14), 0.99 (3H, *d*, *J* = 6.6 Hz, H-15), 1.00 (3H, *s*, H-12), 1.03 (3H, *s*, H-13), 1.12 (1H, *ddd*, *J* = 4.5, 12.7, 14.5 Hz, H-8), 1.29 (1H, *m*, $^w/_2$ = 20.0 Hz, H-2), 1.38 (1H, *m*, $^w/_2$ = 21.5 Hz, H-3), 1.65 (1H, *m*, $^w/_2$ = 20.0 Hz, H-2), 1.81 (1H, *m*, $^w/_2$ = 17.0 Hz, H-5), 1.88 (1H, *m*, $^w/_2$ = 16.5 Hz, H-3), 1.93 (1H, *m*, $^w/_2$ = 15.0 Hz, H-10), 1.94 (1H, *m*, $^w/_2$ = 10.0 Hz, H-1), 1.99 (1H, *m*, $^w/_2$ = 22.0 Hz, H-4), 2.22 (1H, *ddd*, *J* = 5.0, 8.4, 13.8 Hz, H-8), 3.87 (1H, *dt*, *J* = 2.4, 8.4 Hz, H-9).

3.3. (1S,10S)-allo-Aromadendran-9-one (4)

(1*S*,10*S*)-9α-Hydroxy-*allo*-aromadendrane (**2**) (0.432 g, 1.9 mmol) was dissolved in CH_2Cl_2 (25 ml) and Collins reagent ($Cr_2O_3.2C_5H_5N$; 3.619 g, 11.7 mmol) was added over 5 min at room temperature. The reaction mixture was stirred for 3.5 h and $CuSO_4$ (aq) was

added. Extraction with EtOAc (3×50 ml) was followed by concentration in vacuo to obtain (1*S*,10*S*)-*allo*-aromadendran-9-one (**4**). Oil; [α]: −169.9° (*c* = 21.3, $CHCl_3$); IR ν_{max} cm^{-1}: 2955,1709, 1459; EIMS *m*/*z* (rel. int.): 220.1827 (100) $[M]^+$ (calc. 220.1827 for $C_{15}H_{24}O$), 152.1203 (46), 151.1125 (30), 149.1329 (22), 138.1051 (96), 123.1175 (94), 121.1018 (38), 109.1018 (95), 107.0859 (32), 95.0862 (45); ^1H NMR: δ 0.31 (1H, *t*, *J* = 9.5 Hz, H-6), 0.71 (1H, *ddd*, *J* = 6.9, 9.3, 11.0 Hz, H-7), 0.96 (3H, *d*, *J* = 6.8 Hz, H-15), 1.03 (3H, *s*, H-12), 1.08 (3H, *d*, *J* = 6.9 Hz, H-14), 1.10 (3H, *s*, H-13), 1.27 (1H, *m*, $^w/_2$ = 21.0 Hz, H-3), 1.40 (1H, *ddd*, *J* = 6.0, 12.3, 24.2 Hz, H-2), 1.66 (1H, *m*, $^w/_2$ = 18.5 Hz, H-2), 1.785 (1H, *m*, $^w/_2$ = 21.0 Hz, H-3), 1.790 (1H, *m*, $^w/_2$ = 17.5 Hz, H-5), 2.14 (1H, *d*, *J* = 19.0 Hz, H-4), 2.16 (1H, *dd*, *J* = 1.4, 11.0 Hz, H-8), 2.21 (1H, *ddd*, *J* = 4.4, 7.3, 19.8 Hz, H-1), 2.56 (1H, *dd*, *J* = 6.9, 12.2 Hz, H-8), 2.80 (1H, *quintet*, *J* = 6.9 Hz, H-10).

3.4. (1*R*,10*R*)-Aromadendran-9-one (**5**)

(+)-(1*R*,10*R*)-9β-Hydroxyaromadendrane (**3**) (0.430 g, 1.9 mmol) was dissolved in acetone (20 ml) and the solution was cooled to −10 °C (ice-salt). Titration with Jones reagent (6 N, 1.0 ml) followed and the reaction mixture was stirred for 10 min at constant temperature. Ethanol was then added. The mixture was concentrated in vacuo, extracted with EtOAc (3×50 ml), and again concentrated to give (1*R*,10*R*)-aromadendran-9-one (**5**) (0.423 g, 1.9 mol). Needles from acetone, mp 67–68 °C, [α]: −225.4° (*c* = 32.7, $CHCl_3$); IR ν_{max} cm^{-1}: 2953,1703, 1456; EIMS *m*/*z* (rel. int.): 220.1829 (99) $[M]^+$ (calc. 220.1827 for $C_{15}H_{24}O$), 151.1120 (33), 149.1328 (24), 149.0238 (27), 138.1047 (76), 123.1174 (100), 121.1015 (39), 109.1017 (99), 95.0861 (51); ^1H NMR: δ 0.56 (1H, *dd*, *J* = 9.1, 11.8 Hz, H-6), 0.88 (1H, *dd*, *J* = 9.1, 16.6 Hz, H-7), 0.97 (3H, *d*, *J* = 6.9 Hz, H-15), 0.98 (3H, *s*, H-12), 1.01 (3H, *d*, *J* = 6.4 Hz, H-14), 1.12 (3H, *s*, H-13), 1.41 (1H, *m*, $^w/_2$ = 22.9 Hz, H-3), 1.50 (1H, *m*, $^w/_2$ = 16.9 Hz, H-5), 1.53 (1H, *m*, $^w/_2$ = 21.4 Hz, H-2), 1.68 (1H, *ddd*, *J* = 6.1, 9.4, 19.3 Hz, H-1), 1.79 (1H, *m*, $^w/_2$ = 22.4 Hz, H-3), 2.02 (1H, *m*, $^w/_2$ = 22.0 Hz, H-4), 2.03 (1H, *m*, $^w/_2$ = 19.0 Hz, H-2), 2.13 (1H, *ddd*, *J* = 0.8, 9.2, 19.8 Hz, H-8), 2.67 (1H, *dd*, *J* = 8.3, 19.8 Hz, H-8), 3.05 (1H, *ddd*, *J* = 6.5, 10.7, 12.4 Hz, H-10).

3.5. Aromadendra-1,9-diene (**6**)

Squamulosone (0.648 g, 3.0 mmol) was dissolved in THF (10 ml) and lithium aluminium hydride (0.346 g, 9.1 mmol) was added at room temperature with stirring. Stirring was continued for 30 min, H_2SO_4 (6 N) was added and the mixture was extracted with EtOAc (3×50 ml). The extract was concentrated in vacuo and subjected to chromatography using 0.2% EtOAc in petrol to yield aromadendra-1,9-diene (**6**) (0.403 g, 2.0 mmol). Oil; [α]: −25.9° (*c* = 20.1, $CHCl_3$); IR ν_{max} cm^{-1}: 2955, 1657, 1456; UV (EtOH) λ_{max} nm (log ε): 242 (3.6); EIMS *m*/*z* (rel. int.): 202.1721 (2) $[M]^+$ (calc. 202.1721 for $C_{15}H_{26}$), 200.1559 (6), 173.1327 (18), 159.1170 (25), 147.1175 (38), 145.1018 (43), 133.1016 (40), 119.0861 (81), 107.0861 (94), 105.0704 (80), 93.0704 (78), 91.0547 (100); ^1H NMR: δ 0.56 (1H, *dd*, *J* = 9.5, 11.1 Hz, H-6), 0.93 (1H, *dd*, *J* = 9.5, 17.4 Hz, H-7), 1.02 (3H, *s*, H-13), 1.10 (3H, *s*, H-12), 1.07 (3H, *d*, *J* = 6.6 Hz, H-15), 1.83 (3H, *bs*, H-14), 5.50 (1H, *s*, H-9), 5.57 (1H, *t*, *J* = 5.4 Hz, H-2).

3.6. Incubation of (1S,10S)-9α-hydroxy-allo-aromadendrane (**2**)

(1*S*,10*S*)-9α-Hydroxy-*allo*-aromadendrane (**2**) (200 mg) was fed to *C. lunata* in four flasks of each medium as outlined above. Column chromatography resulted in the isolation of the ketone (**4**) (PDB, 92 mg; BEM, 28 mg) and the recovery of the untransformed substrate **2** (PDB, 89 mg; BEM, 116 mg), along with one other metabolite, **7**.

(1*S*,10*S*)-9β-Hydroxy-*allo*-aromadendrane (**7**) (BEM, 4 mg). Spindles from acetone, mp 84–85 °C, [α]: +8.7° (*c* = 67.5, $CHCl_3$); IR ν_{max} cm^{-1}: 3201, 1449; EIMS *m*/*z* (rel. int.): 222.1985 (**7**) $[M]^+$ (calc. 222.1984 for $C_{15}H_{26}O$), 204.4930 (7), 148.4120 (35), 128.4847 (20), 108.5756 (21), 94.6060 (44), 84.5889 (48), 56.8246 (100), 54.8277 (63); ^1H NMR: δ 0.12 (1H, *t*, *J* = 9.3 Hz, H-6), 0.61 (1H, *ddd*, *J* = 6.0, 9.4, 11.8 Hz, H-7), 0.92 (3H, *d*, *J* = 6.8 Hz, H-15), 0.98 (3H, *s*, H-12), 1.045 (3H, *s*, H-13), 1.048 (3H, *d*, *J* = 7.0 Hz, H-14), 1.20 (1H, *m*, $^w/_2$ = 20.0 Hz, H-8), 1.21 (1H, *m*, $^w/_2$ = 25.0 Hz, H-3), 1.48 (1H, *ddd*, *J* = 5.6, 9.1, 23.5 Hz, H-2), 1.64 (1H, *m*, $^w/_2$ = 15.0 Hz, H-5), 1.67 (1H, *m*, $^w/_2$ = 10.0 Hz, H-2), 1.68 (1H, *m* $^w/_2$ = 15.0 Hz, H-10), 1.74 (1H, *m*, $^w/_2$ = 20 Hz, H-3), 1.92 (1H, *m*, $^w/_2$ = 17 Hz, H-1), 2.02 (1H, *m*, $^w/_2$ = 15.2 Hz, H-4), 2.03 (1H, *ddd*, *J* = 1.7, 6.0, 13.6 Hz, H-8), 3.48 (1H, *dt*, *J* = 9.1, 10.7 Hz, H-9).

3.7. Incubation of (1R,10R)-9β-hydroxyaromadendrane (**3**)

(1*R*,10*R*)-9β-Hydroxyaromadendrane (**3**) (200 mg) was fed to *C. lunata* in four flasks of each medium as outlined above. Purification of the extracts resulted in the isolation of the ketone (**5**) (PDB, 5 mg; BEM, 3 mg), and the retrieval of untransformed substrate **3** (PDB, 138 mg; BEM, 166 mg).

3.8. Incubation of (1S,10S)-allo-aromadendran-9-one (**4**)

(1*S*,10*S*)-*allo*-Aromadendran-9-one (**4**) (200 mg) was fed to *C. lunata* in four flasks of each medium as outlined above. The unreacted ketone (**4**) (PDB, 86 mg;

BEM, 64 mg) was obtained along with compounds **2** (PDB, 4 mg) and **7** (PDB, 48 mg; BEM, 68 mg) after the extracts were chromatographed.

3.9. Incubation of (1*R*,10*R*)-aromadendran-9-one (**5**)

(1*R*,10*R*)-Aromadendran-9-one (**5**) (200 mg) was fed to *C. lunata* in four flasks of each medium as outlined above. Column chromatography led to the isolation of the untransformed substrate (**5**) (PDB, 83 mg; BEM, 62 mg), alcohol **3** (PDB, 56 mg; BEM, 27 mg), and one other product, **8**.

(1*R*,10*R*)-13-Hydroxyaromadendran-9-one (**8**) (BEM, 5 mg). Oil; [α]: $-142.5°$ ($c=1.2$, $CHCl_3$); IR ν_{max} cm^{-1}: 3436, 2926, 1742, 1377; EIMS *m*/*z* (rel. int.): 236.1776 (49) $[M]^+$ (calc. 236.1776 for $C_{15}H_{24}O_2$), 167.1072 (29), 163.1487 (15), 161.1330 (10), 151.1123 (15), 147.1174 (11), 135.1174 (9.42), 123.1174 (66), 121.1017 (32), 111.0810 (26), 109.1017 (92), 81.0704 (100); ^{1}H NMR: δ 0.75 (1H, *dd*, $J=9.4$, 11.7 Hz, H-6), 0.99 (3H, *d*, $J=6.8$ Hz, H-15), 1.02 (3H, *d*, $J=6.4$ Hz, H-14), 1.08 (1H, *m*, $^w/_2=19.7$ Hz, H-7), 1.10 (3H, *s*, H-12), 1.42 (1H, *m*, $^w/_2=23.9$ Hz, H-3), 1.55 (1H, *m*, $^w/_2=24.5$ Hz, H-2), 1.56 (1H, *dd*, $J=5.6$, 12.8 Hz, H-5), 1.71 (1H, *ddd*, $J=6.0$, 9.4, 19.2 Hz, H-1), 1.81 (1H, *m*, $^w/_2=22.3$ Hz, H-3), 2.05 (1H, *m*, $^w/_2=18.7$ Hz, H-2), 2.05 (1H, *m*, $^w/_2=18.7$ Hz, H-4), 2.17 (1H, *ddd*, $J=1.2$, 9.1, 18.9 Hz, H-8), 2.70 (1H, *dd*, $J=8.2$, 19.7 Hz, H-8), 3.05 (1H, *ddd*, $J=6.0$, 10.4, 12.9 Hz, H-1), 3.37 (1H, *d*, $J=11.0$ Hz, H-13), 3.54 (1H, *d*, $J=10.9$ Hz, H-13).

3.10. Incubation of aromadendra-1,9-diene (**6**)

Aromadendra-1,9-diene (**6**) (200 mg) was fed to *C. lunata* in four flasks of each medium as outlined above. Purification of the extracts gave the compounds listed below.

2β-Hydroxyaromadendr-1(10)-en-9-one (**9**) (PDB, 4 mg; BEM, 12 mg). Cubes from acetone, mp 80–82 °C, [α]: $-186°$ ($c=3.2$, $CHCl_3$) [lit. mp 85–86 °C; [α]: $-186°$ ($c=6.3$, $CHCl_3$) (Collins et al., 2001)]; IR ν_{max} cm^{-1}: 3418, 2928, 1654; UV ($CHCl_3$) λ_{max} nm (log ε): 256 (3.0); ^{1}H NMR: δ 0.68 (1H, *ddd*, $J=5.1$, 9.4, 11.9 Hz, H-7), 0.81 (1H, *dd*, $J=10.4$, 11.0 Hz, H-6), 1.05 (3H, *d*, $J=6.8$ Hz, H-15), 1.07 (3H, *s*, H-13), 1.19 (3H, *s*, H-12), 1.57 (1H, *dt*, $J=4.9$, 12.6 Hz, H-3), 1.93 (1H, *dd*, $J=5.8$, 13.4 Hz, H-3), 1.94 (3H, *d*, $J=1.6$ Hz, H-14), 2.37 (1H, *dd*, $J=11.7$, 15.7 Hz, H-8), 2.68 (1H, *m*, $^w/_2=21.2$ Hz, H-4), 2.73 (1H, *dd*, $J=9.1$, 19.1 Hz, H-5), 2.84 (1H, *dd*, $J=5.1$, 14.9 Hz, H-8), 4.92 (1H, *d*, $J=6.9$ Hz, H-9).

9β,10β-Dihydroxyaromadendr-1-ene (**10**) (PDB, 4 mg; BEM, 6 mg). Oil [α]: $+31.5°$ ($c=5.2$, $CHCl_3$); IR ν_{max} cm^{-1}: 3392, 1376, 1032; EIMS *m*/*z* 236.1776 (31) $[M]^+$ (calc. 218.1671 for $C_{15}H_{22}O$), 218.1671 (22), 203.1436 (16), 175.1123 (23), 149.0966 (26), 147.1174 (37), 95.0861 (100); ^{1}H NMR: δ 0.41 (1H, *t*, $J=9.6$ Hz, H-6), 0.50 (1H, *ddd*, $J=6.0$, 9.4, 11.2 Hz, H-7), 1.03 (3H, *s*, H-13), 1.05 (3H, *s*, H-12), 1.06 (3H, *d*, $J=5.4$ H-15), 1.51 (3H, *s*, H-14), 1.72 (1H, *dd*, $J=11.1$, 14.0 Hz, H-8), 1.78 (1H, *dd*, $J=2.8$, 6.4 Hz, H-8), 2.03 (1H, *m*, $^w/_2=20.5$ Hz, H-3), 2.26 (1H, *m*, $^w/_2=16.1$ Hz, H-3), 2.37 (1H, *m*, $^w/_2=20.5$ Hz, H-5), 2.39 (1H, *m*, $^w/_2=20.5$ Hz, H-4), 3.39 (1H, *d*, $J=10.1$ Hz, H-9), 5.70 (1H, *dd*, $J=1.8$, 3.1 Hz, H-2).

2β,9β-Dihydroxyaromadendr-1(10)-ene (**11**) (PDB, 10 mg; BEM, 8 mg). Cubes from an acetone:chloroform (3:1, v:v) mixture, mp 137–138 °C, [α]: $-21.2°$ ($c=12.1$, $CHCl_3$); IR ν_{max} cm^{-1}: 3355, 2954,1456; EIMS *m*/*z* (rel. int.): 218.1671 (12) $[M–H_2O]^+$ (calc. 218.1671 for $C_{15}H_{22}O$), 203.1436 (11), 195.1385 (17), 191.1072 (14), 176.1201 (52), 159.1174 (23), 59.0497 (100); ^{1}H NMR: δ 0.45 (1H, *ddd*, $J=4.6$, 9.3, 12.0 Hz, H-7), 0.53 (1H, *dd*, $J=9.3$, 11.0 Hz, H-6), 0.99 (3H, *d*, $J=6.8$ Hz, H-15), 1.01 (3H, *s*, H-13), 1.09 (3H, *s*, H-12), 1.37 (1H, *dt*, $J=4.7$, 13.3 Hz, H-3), 1.57 (1H, *dt*, $J=12.0$, 13.7 Hz, H-8), 1.79 (1H, *dd*, $J=5.8$, 13.3 Hz, H-3), 1.93 (3H, *d*, $J=1.1$ Hz, H-14), 2.13 (1H, *dt*, $J=5.0$, 13.6 Hz, H-8), 2.53 (1H, *m*, $^w/_2=21.6$ Hz, H-5), 2.62 (1H, *t*, $J=9.6$ Hz, H-4), 4.10 (1H, *dd*, $J=5.0$, 11.7 Hz, H-9), 4.74 (1H, *d*, $J=4.7$ Hz, H-2).

2β,9α-Dihydroxyaromadendr-1(10)-ene (**12**) (PDB, 9 mg; BEM, 9 mg). Oil; [α]: $+10.6°$ ($c=8.0$, $CHCl_3$); IR ν_{max} cm^{-1}: 3370, 2952,1375; EIMS *m*/*z* (rel. int.): 218.1671 (31) $[M–H_2O]^+$ (calc. 236.1776 for $C_{15}H_{24}O_2$), 216.1514 (9), 209.1542 (5), 207.1385 (6), 205.1229 (6), 203.1436 (20), 55.055 (100); ^{1}H NMR: δ 0.70 (1H, *m*, $^w/_2=9.5$ Hz, H-7), 0.79 (1H, *dd*, $J=5.1$, 9.5 Hz, H-6), 0.98 (3H, *d*, $J=6.3$ Hz, H-15), 1.01 (3H, *s*, H-13), 1.10 (1H, *s*, H-12), 1.78 (1H, *dd*, $J=3.8$, 15.2 Hz, H-3), 1.91 (3H, *bs*, H-14), 2.57 (1H, *dd*, $J=2.5$, 8.5 Hz, H-5), 4.30 (1H, *t*, $J=2.5$ Hz, H-9), 4.74 (1H, *d*, $J=4.4$ Hz, H-2).

(1*S*,10*S*)-1α,2β,9β,10β-Tetrahydroxyaromadendrane (**13**) (PDB, 3 mg; BEM, 2 mg). Oil; [α]: $+20.0°$ ($c=2.1$, $CHCl_3$); IR ν_{max} cm^{-1}: 3402, 1455; EIMS *m*/*z* (rel. int.): 234.1618 (10) $[M–2H_2O]^+$ (calc. 234.1620 for $C_{15}H_{26}O_4$), 205.1592 (9), 191.1436 (11), 177.1279 (13), 175.1123 (26), 163.1123 (28), 161.0966 (17), 149.0966 (45), 135.0810 (100); ^{1}H NMR: δ 0.16 (1H, *dd*, $J=9.1$, 11.2 Hz, H-6), 0.88 (1H, *m*, $^w/_2=8.0$ Hz, H-7), 0.90 (3H, *d*, $J=4$ Hz, H-15), 0.98 (3H, *s*, H-12), 1.08 (3H, *s*, H-13), 1.20 (3H, *s*, H-14), 1.24 (1H, *m*, $^w/_2=10.0$ Hz, H-8), 1.37 (1H, *m*, $^w/_2=8.0$ Hz, H-3), 1.75 (1H, *m*, $^w/_2=8.2$ Hz, H-4), 2.03 (1H, *m*, $^w/_2=12.5$ Hz, H-3), 2.04 (1H, *m*, $^w/_2=10.0$ Hz, H-5), 2.40 (1H, *ddd*, $J=5.2$, 7.1, 15.0 Hz, H-8), 3.21 (1H, *dd*, $J=5.7$, 7.1 Hz, H-9), 3.50 (1H, *bs*, H-2).

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