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Biotransformation of cadina-4,10(15)-dien-3-one and 3α-hydroxycadina-4,10(15)-diene 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

Cadina-4,10(15)-dien-3-one (**1**) was metabolised by *Curvularia lunata* ATCC 12017 in two different growth media to give three metabolites, one of which, 12-hydroxycadina-4,10(15)-dien-3-one (**4**), was new. Incubation of 3α-hydroxycadina-4,10(15)-diene (**2**) with the fungus produced three new analogues, namely, (4*S*)-1α,3α-dihydroxycadin-10(15)-ene (**5**), 3α,14-dihydroxycadina-4,10(15)-diene (**6**) and 3α,12-dihydroxycadina-4,10(15)-diene (**7**).

Keywords: *Curvularia lunata* ATCC 12017; Cadinane; Biotransformation; Hydroxylation; *Hyptis verticillata*; Sesquiterpene

1. Introduction

Cadina-4,10(15)-dien-3-one (**1**) is a naturally occurring sesquiterpene that was isolated from Jamaican variety of the medicinal plant *Hyptis verticillata* (Porter et al., 1995). The metabolism of **1** along with the synthetically derived allylic alcohol, 3α-hydroxycadina-4,10(15)-diene (**2**), were previously investigated using the fungus *Beauveria bassiana* ATCC 7159 (Buchanan and Reese, 2000). This resulted in the isolation of nine analogues that were mainly derived from redox reactions.

In an ongoing programme to examine the transformation of terpenes by fungi (Hanson et al., 1994; Buchanan and Reese, 2000), with the aim of generating metabolites with enhanced biological activity cadina-4,10(15)-dien-3-one (**1**), an insecticidal and acaricidal agent, and its synthetic derivative **2** were fed to *Curvularia lunata* ATCC 12017 (synonyms: IMI 61535, CBS 215.54, NRRL 2380). This fungus was chosen because of its established history in biotransformation. It is especially known for the 11β-hydroxylation of steroids (Ohlson et al., 1980; Chen and Wey, 1990; Holland, 1992; Mazumder et al., 1985). Few sesquiterpenes have been transformed, however (Azerad, 2000; Garcia-Granados et al., 1991; de Lima et al., 1999; Amate et al., 1990). It is our intention to further investigate the metabolism of a number of sesquiterpene skeleta with this microorganism. Previously the conversion of a cedrane (Collins and Reese, 2001) and an aromadendrane (Collins et al., 2001) were studied in two different liquid growth media, *viz*, potato dextrose broth, PDB (Chen and Wey, 1990), and a beef extract medium, BEM (Garcia-Granados et al., 1991). In this study three products (one new) were formed from **1** after growth in PDB, two of which were also obtained with the use of BEM. The incubation of substrate **2** with *C. lunata* yielded three metabolites, all of which were novel.

2. Results and discussion

The incubation of cadina-4,10(15)-dien-3-one (**1**) with *C. lunata* in PDB produced three metabolites that were identified as 3α-hydroxycadina-4,10(15)-diene (**2**), 4*S*-3α-hydroxycadin-10(15)-ene (**3**) and the hitherto unreported 12-hydroxycadina-4,10(15)-dien-3-one (**4**). When **1** was incubated in BEM only **2** and **4** were isolated. Metabolites **2** and **3** were identified by comparison of their spectral data with those in the literature.

HR(EI)MS data for **4** suggested a molecular formula of $C_{15}H_{22}O_2$ ($[M]^+ = 234.1614$) that was consistent with the presence of a new hydroxyl group (ν_{max} 3460 cm^{-1}) within the molecule. Retention of the enone system in **4** was also apparent (1712 and 1664 cm^{-1}). The appearance

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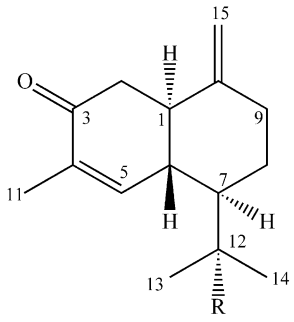
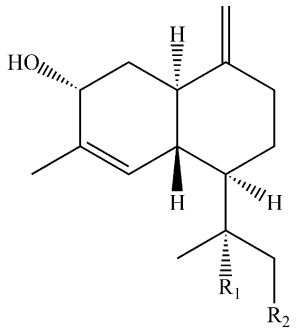
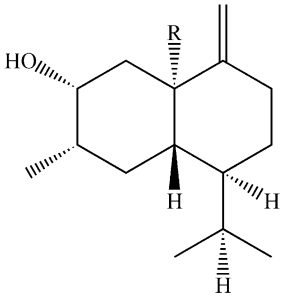
of the signal at δ 74.2 as a nonprotonated carbon together with the absence of a methine at δ 26.4 strongly suggested that hydroxylation had occurred at C-12. This assignment was supported further by the large downfield shifts observed for C-7, C-13 and C-14 when compared with **1**.



1: R = H
4: R = OH



2: $R_1 = R_2 = H$
6: $R_1 = H$, $R_2 = OH$
7: $R_1 = OH$, $R_2 = H$



3: R = H
5: R = OH

Analogue **2,** synthesised from **1**, was incubated with *C. lunata* in PDB to give two metabolites that were ascertained to be (4*S*)-1α,3α-dihydroxycadin-10(15)-ene (**5**) and 3α,14-dihydroxycadina-4,10(15)-diene (**6**). The fermentation of **2** in BEM led to the isolation of **5** in addition to one other metabolite that was determined to be 3α,12-dihydroxycadina-4,10(15)-diene (**7**). The isolation of terpenes **5**–**7** has not been reported previously.

Data from the HR(EI)MS of **5** suggested a molecular formula of $C_{15}H_{24}O_2$ ($[M]^+ = 238.1567$). The exocyclic double bond was present (δ_H 4.51 and 4.68), however, reduction of the α,β-unsaturated system to the saturated alcohol (δ_C 68.8) was evident. The other oxygen function, also a hydroxyl (3445 cm^{-1}), was placed at C-1 based on its high resonance value of 88.6 ppm which suggested that it resided at a ring junction. A comparison of the carbon resonances of **5** with those of **3** reinforced the proposed structure. The probable sequence leading to the formation of this compound involves oxidation of the secondary alcohol to the carbonyl. The reduction of the olefin of the newly formed α,β-unsaturated system then preceeded reduction of the carbonyl back to the 3α-hydroxy product (**5**). The point at which C-1 hydroxylation occurred can only be speculated.

The ^{13}C NMR spectrum of **6** contained two oxygen-bearing carbon atoms that were revealed by DEPT experiments to be a methine (δ_C 71.1) and a methylene (δ_C 66.9). The former resonance was assigned to C-3 and coincided with a reduction of the ketone to form an allylic alcohol (δ_H 3.54 and 5.61). The latter was placed at C-14 based on the chemical shifts of C-12 and C-13 that were consistent with hydroxylation at this point. Compound **7** differed from **6** in that hydroxylation had occurred at C-12.

In summary **1** was metabolised by *C. lunata* to produce two analogues (**2** and **4**) in BEM and an additional metabolite (**3**) in PDB. 12-Hydroxycadina-4,10(15)-dien-3-one (**4**), the only product of hydroxylation, was novel. The incubation of substrate **2** with *C. lunata* under both growth conditions led to the isolation of three novel metabolites, **5**, **6** and **7**. Hydroxylation occurred at C-1, C-12 and C-14. C-1 hydroxylation was common to both media.

3. Experimental

^1H and ^{13}C NMR spectra were generated in deuterated chloroform at 200 and 50 MHz respectively using a Bruker AC200 instrument. Tetramethylsilane (TMS) was used as the internal standard. ^{13}C NMR assignments are listed in Table 1. Melting points were measured on a Thomas-Hoover capillary melting point apparatus. Infrared data were obtained on a Perkin Elmer FTIR Paragon 1000 instrument using KBr disks. The 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 Kratos MS50 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. *Curvularia lunata* ATCC 12017 was obtained from the American Type Culture Collection, Rockville, MD, USA. Petrol refers to the petroleum fraction boiling between 60 and 80 °C.

3.1. Generation of substrates

Hyptis verticillata plant material was collected in St Andrew, Jamaica. A voucher specimen was deposited in the Botany Herbarium, UWI (accession number 33483). The fresh green leaves and stems (7.6 kg) were chopped and extracted twice with CH_2Cl_2 (30 l) at room temperature. The extracts were pooled and concentrated in vacuo to produce a dark green gum (24.4 g, 0.32%).

Table 1
C-13 NMR chemical shifts for cadinanes **1**–**7**

Carbon	Compounds						
	1	**2**	**3**	**4**	**5**	**6**	**7**
1	45.31	46.3	45.5	45.9	88.6	44.9	46.2
2	41.4	36.2	36.7	41.4	43.1	36.3	36.2
3	199.9	71.0	70.4	200.0	68.8	71.1	70.7
4	135.5	136.8	35.8	134.3	39.2	137.2	135.9
5	146.4	125.7	31.9	149.4	35.2	125.4	128.4
6	45.29	43.6	39.0	44.6	45.1	43.7	43.2
7	45.1	45.4	47.8	51.0	47.7	41.4	52.5
8	26.3	26.4	26.3	31.1	26.0	26.8	31.2
9	35.5	35.9	36.4	35.4	36.0	35.9	35.9
10	149.8	151.6	153.3	150.1	151.0	151.2	151.2
11	16.0	19.3	18.6	16.1	15.0	19.3	19.3
12	26.4	26.3	26.0	74.2	26.5	34.8	73.1
13	15.2	15.0	15.0	23.7	15.2	10.1	23.8
14	21.5	21.4	21.6	32.7	21.5	66.9	32.4
15	105.3	103.7	103.4	105.5	104.5	104.2	104.9

The crude extract was chromatographed on silica gel using 3% EtOAc in petrol to give eight major fractions (A–H). The moderately non-polar fraction B (7.6 g) was subjected to further purification by column chromatography using 0.5% EtOAc in petrol. This led to the isolation of a cadina-4,10(15)-dien-3-one (**1**) (1.5 g, 0.02%).

*3.1.1. Cadina-4,10(15)-dien-3-one (**1**)*

Needles from acetone, mp 74–75 °C; $[\alpha]_D^{25}$: +134° (c = 10.3, $CHCl_3$) [lit. mp 79–80 °C, $[\alpha]_D^{25}$: +127° (c = 1.6, $CHCl_3$) (Porter et al., 1995)]; IR ν_{max} cm^{-1} 2950, 1680, 890; ^1H NMR: δ 0.80 (3H, *d*, J = 12 Hz, H-14), 1.00 (3H, *d*, J = 12 Hz, H-13), 1.80 (3H, *m*, $w/2$ = 17 Hz, H-11), 2.25 (1H, *m*, $w/2$ = 38 Hz, H-12), 4.47 (1H, *s*, H-15), 4.73 (1H, *s*, H-15), 6.82 (1H, *s*, H-5).

*3.2. 3α-Hydroxycadina-4,10(15)-diene (**2**)*

Cadina-4,10(15)-dien-3-one (0.7278 g, 3.34 mmol) was dissolved in tetrahydrofuran (25 ml) and lanthanum chloride (1.141 g, 4.68 mmol) was added. The mixture was stirred at room temperature for 45 min after which lithium aluminium hydride (0.502 g, 13.21 mmol) was added. The reaction was left for 30 min, was neutralised with dilute hydrochloric acid, and extracted with diethyl ether (3×50 ml) and ethyl acetate (3×50 ml). The combined organic extracts were dried with magnesium sulfate and concentrated in vacuo to give 3α-hydroxycadina-4,10(15)-diene (**2**) (0.4643 g, 2.11 mmol): Needles, mp 113–114 °C; $[\alpha]_D^{25}$: −71.0° (c = 4.6, $CHCl_3$) [lit. mp 115–117 °C (Buchanan and Reese, 2000)]; IR ν_{max} cm^{-1} 3402, 3348, 2966, 1652, 1044, 896; ^1H NMR δ 0.74 (3H, *d*, J = 7.6 Hz, H-13), 0.99 (3H, *d*, J = 7.6 Hz, H-14), 1.77 (3H, *d*, J = 0.8 Hz, H-11), 4.23 (1H, *bt*, J = 9.3 Hz, H-3), 4.52 (1H, *d*, J = 0.9 Hz, H-15), 4.69 (1H, *d*, J = 0.9 Hz, H-15), 5.60 (1H, *d*, J = 0.9 Hz, H-5).

3.3. Feeding protocol

The fungus was maintained on PDA slants. PDB was comprised of potato dextrose broth (24 g l^{-1}) (Chen and Wey, 1990). BEM was composed per litre of peptone (1 g), yeast extract (1 g), beef extract (1 g) and glucose (5 g) (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 incubated at 200 rpm at 27 °C. An EtOAc solution (2 ml) containing 10% of the total mass of the substrate was fed 24 h after inoculation. The remaining 20, 30 and 40% of the substrate was fed at 36, 48 and 60 h respectively after inoculation. The fermentation was allowed to proceed for 10 days after the last feed. The pH was measured 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.

*3.4. Incubation of cadina-4,10(15)-dien-3-one (**1**)*

Cadina-4,10(15)-dien-3-one (**1**) (200 mg) was fed to *C. lunata* in 20 flasks of each medium as outlined above. After 10 days the fungus was harvested to give broth extracts (0.465 and 0.501 g) and mycelial extracts (0.426 and 0.377 g) from PDB and BEM respectively. Analysis of both extracts by TLC indicated the presence of biotransformed compounds. The broth and mycelial extracts of each fermentation were combined and subjected to column chromatography using increasing concentrations of EtOAc in petrol.

*3.4.1. 3α-Hydroxycadina-4,10(15)-diene (**2**) (PDB, 16 mg)*

Identified by comparison with authentic material.

*3.4.2. 4S-3α-Hydroxycadin-10(15)-ene (**3**) (PDB, 16 mg; BEM, 4 mg)*

Cubes from acetone, mp 132–134 °C [lit. oil (Buchanan and Reese, 2000)]; $[\alpha]_D^{25}$: +19.0° (c = 2.6, $CHCl_3$); IR ν_{max} cm^{-1} 3299, 1676, 1468, 1061; ^1H NMR δ 0.69 (3H, *d*, J = 7.0 Hz, H-14), 0.90 (3H, *d*, J = 7.0 Hz, H-14), 0.98 (3H, *d*, J = 7.0 Hz, H-11), 2.02 (3H, *m*, $w/2$ = 21.3 Hz, H-12), 3.92 (1H, *bd*, J = 2.8 Hz, H-3), 4.49 (1H, *bs*, H-15), 4.63 (1H, *d*, J = 1.6 Hz, H-15).

*3.4.3. 12-Hydroxycadina-4,10(15)-dien-3-one (**4**) (PDB, 4 mg; BEM, 5 mg)*

Oil; $[\alpha]_D^{25}$: −14.0° (c = 2.0, $CHCl_3$); IR ν_{max} cm^{-1} 3460, 2932, 1712, 1664, 1452, 1371; UV ($CHCl_3$) λ_{max} nm (log ε) 243 (3.18); EIMS m/z (rel. int.) 234.1614 (9) $[M]^+$, 216.1511 (100), 177.1281 (29), 176.1214 (48), 173.0979 (48), 161.0983 (47), 148.1269 (23), 147.1199 (8), 133.1045 (20); ^1H NMR δ 1.22 (3H, *s*, H-13), 1.25

(3H, *s*, H-14), 1.32 (3H, *s*, H-11), 1.80 (1H, *dd*, $J=1.2$, 3.2 Hz, H-8), 2.10 (2H, *m*, $w/2=26.0$ Hz, H-6), 2.40 (1H, *m*, $w/2=15.5$ Hz, H-1), 4.56 (1H, *s*, H-15), 4.76 (1H, *s*, H-15), 7.90 (1H, *d*, $J=7.0$ Hz, H-5).

3.5. Incubation of 3α-hydroxycadina-4,10(15)-diene (2)

3α-Hydroxycadina-4,10(15)-dien-3-one (**2**) (200 mg) was fed to *C. lunata* as described above. After 10 days the fungus was harvested to give broth extracts (0.420 and 0.432 g) and mycelial extracts (0.419 and 0.402 g) from PDB and BEM respectively. Analysis of both extracts by TLC indicated the presence of biotransformed compounds. The broth and mycelial extracts of each fermentation were combined and subjected to column chromatography using increasing concentrations of EtOAc in petrol.

3.5.1. (4S)-1α,3α-Dihydroxycadin-10(15)-ene (5) (PDB, 6 mg; BEM, 4 mg)

Oil; $[\alpha]_D^{25}$: -33.2° ($c=1.9$, $CHCl_3$); IR ν_{max} cm^{-1} 3445, 2936, 1664, 1388; EIMS *m/z* (rel. int.) 238.1567 (100) $[M]^+$, 204.1875 (45), 161.1330 (54), 133.1015 (17), 107.0860 (33), 105.0704 (44); ^1H NMR δ 0.71 (3H, *d*, $J=7.0$ Hz, H-13), 0.86 (3H, *d*, $J=6.3$ Hz, H-11), 0.92 (3H, *d*, $J=7.0$ Hz, H-14), 2.61 (1H, *dd*, $J=4.1$, 9.2 Hz, H-9), 4.51 (1H, *m*, w/2 = 9.2 Hz, H-15), 4.68 (1H, *s*, H-15), 6.39 (1H, *s*, H-5).

3.5.2. 3α,14-Dihydroxycadina-4,10(15)-diene (6) (PDB, 3 mg)

Oil; $[\alpha]_D^{25}$: $+8.8^\circ$ ($c=4.3$, $CHCl_3$); IR ν_{max} cm^{-1} 3445, 2978, 1693, 1380; EIMS *m/z* (rel. int.) 236.1780 (12) $[M]^+$, 218.1671 (32), 177.1278 (18), 163.1127 (28), 162.1410 (39), 159.1177 (100), 147.1175 (41), 133.1016 (35); ^1H NMR δ 0.78 (3H, *d*, $J=7.0$ Hz, H-13), 1.79 (3H, *s*, H-11), 2.35 (1H, *m*, w/2 = 15.5 Hz, H-6), 3.54 (1H, *d*, $J=8.2$ Hz, H-3), 4.58 (1H, *s*, H-15), 4.70 (1H, *d*, $J=7.9$ Hz, H-15), 5.61 (1H, *s*, H-5).

3.5.3. 3α,12-Dihydroxycadina-4,10(15)-diene (7) (BEM, 7 mg)

Oil; $[\alpha]_D^{25}$: $+64.2^\circ$ ($c=1.9$, $CHCl_3$); IR ν_{max} cm^{-1} 3447, 2887, 1664, 1380; EIMS *m/z* (rel. int.) 236.1770 (6) $[M]^+$, 218.1669 (45), 175.1119 (86), 160.1256 (93), 157.1022 (42), 147.1172 (13), 133.1011 (15), 59.0496 (100); ^1H NMR δ 1.18 (3H, *s*, H-13), 1.25 (3H, *s*, H-14), 1.79 (3H, *s*, H-11), 2.35 (1H, *m*, w/2 = 15.2 Hz, H-6), 3.46 (1H, *t*, $J=7.0$ Hz, H-5), 3.61 (1H, *m*, w/2 = 15.2 Hz, H-3), 4.56 (1H, *m*, w/2 = 10.1 Hz, H-15), 4.7 (1H, *m*, w/2 = 10.1 Hz, H-15).

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References

Amate, Y., Breton, J., Garcia-Granados, A., Martinez, A., Onorato, M.E., de Buruaga, A.S., 1990. Synthesis, biotransformation and stereochemistry of 6β-sesquiterpene lactones: synthesis of 6β-artepaulin, 11,13-dihydro-6β-tuberiferin, 5,15-dihydro-6β-oopodin, 4-epi-6β-vulgarin and 6β-vulgarin. Tetrahedron 46, 6939–6950.

Azerad, R., 2000. Regio- and stereoselective microbial hydroxylation of terpenoid compounds. In: Patel, R.N. (Ed.), Stereoselective Biocatalysis. Marcel Dekker, New York, pp. 153–180.

Buchanan, G.O., Reese, P.B., 2000. Biotransformation of cadinane sesquiterpenes by *Beauveria bassiana* ATCC 7159. Phytochemistry 54, 39–45.

Collins, D.O., Buchanan, G.O., Reynolds, W.F., Reese, P.B., 2001. Biotransformation of squamulosone by *Curvularia lunata* ATCC 12017. Phytochemistry 57, 377–383.

Collins, D.O., Reese, P.B., 2001. Biotransformation of cedrol by *Curvularia lunata* ATCC 12047. Phytochemistry 56, 417–421.

Chen, K., Wey, H., 1990. Dissolution enzyme kinetics of 11β-hydroxylation of cortexolone by *Curvularia lunata*. Enzyme Microbiology and Technology 12, 616–621.

de Lima, D.P., Carnell, A.J., Roberts, S.M., 1999. Microbial transformation of (+)-10β,14-dihydroxy-allo-aromadendrane and (-)-allo-aromadendrone. Journal of Chemical Research (Synopses) 396–397.

Garcia-Granados, A., Martinez, A., Onorato, M.E., Rivas, F., Arias, J.M., 1991. Chemical-microbial synthesis of 6β-eudesmanolides by *Curvularia lunata* cultures from eudesmanes with functions at C-1 and C-6. Tetrahedron 47, 91–102.

Hanson, J.R., Reese, P.B., Takahashi, J.A., Wilson, M.R., 1994. Biotransformation of some stemodane diterpenoids by *Cephalosporium aphidicola*. Phytochemistry 36, 1391–1393.

Holland, H.L., 1992. Organic Synthesis with Oxidative Enzymes. VCH Publishers, New York, pp. 86–95.

Mazumder, T.K., Sonomoto, K., Tanaka, A., Fukui, S., 1985. Sequential conversion of cortexolone to prednisolone by immobilized mycelia of *Curvularia lunata* and immobilized cells of *Arthrobacter simplex*. Applied Microbiology and Biotechnology 21, 154–161.

Ohlson, S., Flygare, S., Larsson, P., Mosbach, K., 1980. Steroid hydroxylation using immobilized spores of *Curvularia lunata* germinated in situ. European Journal of Applied Microbiology 10, 1–9.

Porter, R.B.R., Reese, P.B., Williams, L.A.D., Williams, D.J., 1995. Acaricidal and insecticidal activities of cadina-4,10(15)-dien-3-one. Phytochemistry 40, 735–738.