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Biotransformation of cadinane sesquiterpenes by *Beauveria bassiana* ATCC 7159

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Dedicated to Dr. Earle V. Roberts in celebration of his service of over 30 years to the Chemistry Department, University of the West Indies, Mona Campus

Abstract

Incubation of cadina-4,10(15)-dien-3-one with *Beauveria bassiana* ATCC 7159 has resulted in the production of nine novel sesquiterpenes. These metabolites were identified as (4S)-cadin-10(15)-en-3-one, (4S)-3α-hydroxycadin-10(15)-ene, (4R)-3α-hydroxycadin-10(15)-ene, (4S)-3β-hydroxycadin-10(15)-ene, (4S)-3β-hydroxycadina-10(15),12(14)-diene, (4S)-13-hydroxycadin-10(15)-en-3-one, (4S)-12-hydroxycadin-10(15)-en-3-one, (4R)-3β,14-dihydroxycadin-10(15)-ene and 3α-hydroxycadina-4,10(15)-diene. The allylic alcohol 3α-hydroxycadina-4,10(15)-diene was also biotransformed to afford cadina-4,10(15)-dien-3-one, (4S)-cadin-10(15)-en-3-one and (4S)-12-hydroxycadin-10(15)-en-3-one. The insecticidal potential and phytotoxicity of the isolated metabolites have been evaluated.

Keywords: *Beauveria bassiana* ATCC 7159; Deuteromycotina; Biotransformation; Cadinane; Sesquiterpene; *Hyptis verticillata*

1. Introduction

The deuteromycete *Beauveria bassiana* ATCC 7159, the fungus which is responsible for the muscaridine disease in insects, has been used to effect the bioconversion of many substrates including alkaloids, steroids and terpenes. This strain, formerly known as *B. sulfurescens* or *Sporotrichum sulfurescens*, has also been used to effect the reduction of various carbonyl compounds (Davies et al., 1989). α,β-Unsaturated xenobiotes, e.g. (±)-carvone have been reduced to their corresponding saturated ketones or alcohols depending on the length of the incubation period (Kergomard et al., 1982). *B. bassiana* is also known to selectively hydroxylate non-activated carbon atoms (Goswami et al., 1987; Hammoumi et al., 1991; Lamare et al., 1991; Holland, 1992).

We report here the results of the action of *B. bassiana* ATCC 7159 on the naturally occurring sesquiterpene cadina-4,10(15)-dien-3-one (**1**) and its synthetic reduction product, 3α-hydroxycadina-4,10(15)-diene (**2**). The former was isolated in large quantity from the green leaves and twigs of the plant *Hyptis verticillata*. This compound has been shown to possess insecticidal activity against the sweet potato weevil *Cylas formicarius elegantulus* and acaricidal potential against the cattle tick *Boophilus microplus* (Porter et al., 1995). In an effort to produce new functionalised analogues, with improved biological activity, the fungal bioconversion of these cadinanes was investigated.

2. Results and discussion

Cadina-4,10(15)-dien-3-one (**1**) was isolated from

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Hyptis verticillata (Jacq.) (Porter et al., 1995). The previously reported ^{13}C NMR assignments (Porter et al., 1995) for C-11 and C-14 have been revised to 16.0 and 21.5 ppm, respectively (Table 1). This reversal was arrived at after careful analysis of the results obtained from 2D NMR experiments as well as comparison of the carbon data with those from a number of cadinanes. The allylic alcohol **2** was prepared by stereoselective reduction of the naturally occurring ketone by lithium aluminium hydride in the presence of lanthanum chloride (Neef et al., 1982). 3α-Hydroxycadina-4,10(15)-diene was identified by the presence of an M^+ peak at *m/z* 220.1824 ($C_{15}H_{24}O$) in the high resolution electron impact mass spectrum (HREIMS). The axial configuration of the hydroxyl was determined from the multiplicity of the H-3 signal.

Two saturated alcohols, **3** and **4**, were also obtained as minor products. The HREIMS suggested a molecular formula of $C_{15}H_{26}O$ ($M^+ = 222.1981$ and 222.1920). The stereochemistry of the methyl substituent at C-4 was determined from the position of its ^{13}C NMR signal. An equatorial methyl had a resonance value greater than 14.0 ppm. On the other hand, a peak at less than 12.0 ppm fixed the group as axial. The stereochemistry of the hydroxyls was established by noting the multiplicity of H-3, as well as the coupling constants. The ^1H NMR of **3** revealed a signal at δ 3.92 (1H, *bd*, $J = 2.82$ Hz), indicative of an equatorial proton. Similarly, **4** possessed an equatorial proton. The two minor products were thus (4S)-3α-hydroxycadin-10(15)-ene (**3**) and (4R)-3α-hydroxycadin-10(15)-ene (**4**).

Generally the EI mass spectra of the three hydroxy compounds synthesised contained fragment ions indicative of the elimination of water followed by the loss of the isopropyl group. In some instances, the loss of a molecule of water was accompanied by a retro-Diels-Alder reaction in ring A. These fragmentation patterns were also evident in the fungal metabolites.

Sesquiterpene **1** was incubated with *Beauveria bassiana* using a pulse feeding protocol (Goodhue et al., 1986). Several metabolites were detected; however, only four were obtained in sufficient quantities for characterisation. The HREIMS of the major metabolite, (4S)-cadin-10(15)-en-3-one, (**5**) indicated a molecular formula of $C_{15}H_{24}O$ ($[M]^+ = 220.1826$). The IR spectrum showed a C═O stretch at 1715 cm^{-1}, indicating a loss of conjugation. The stereochemistry of the C-4 methyl substituent was α since its resonance signal appeared at 14.3 ppm. The S configuration of the product is at variance with that seen in the reduction of other cyclic α,β-unsaturated ketones by *B. bassiana* (Lamare et al., 1991). Saturated alcohols **3** and **6** were also isolated. Metabolite **3** had been obtained previously as a minor product in the reduction of **1**. Terpene **6** was identified as (4S)-3β-hydroxycadin-10(15)-ene. The most polar compound was identified as a dihydroxycadinane. From the ^{13}C and DEPT NMR experiments only two methyl carbons were present in the molecule. There was also a methylene bearing oxygen resonating at 66.8 ppm. A comparison of the NMR spectrum of **7** with that of the substrate clearly indicated that reduction of the enone as well as hydroxylation at C-14 had occurred.

Encouraged by the results obtained, a larger scale fermentation of the sesquiterpene (**1**) was carried out in order to improve the yields of other products. The incubation period was increased from 9 to 13 days. Metabolites **3**, **5** and **6** were again found. Additionally five new transformed terpenes were isolated. (4R)-3α-

Table 1
^{13}C NMR chemical shift assignments for cadinane metabolites (**1**–**10**)[a]

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
C-1	45.3 (1)	46.3 (1)	45.5 (1)	45.2 (1)	45.0 (1)	45.3 (1)	44.4 (1)	45.0 (1)	45.6 (1)	44.4 (1)
C-2	41.4 (2)	36.2 (2)	36.7 (2)	36.4 (2)	44.3 (2)	37.6 (2)	35.9 (2)	38.1 (2)	44.4 (2)	44.4 (2)
C-3	199.9 (0)	71.0 (1)	70.4 (1)	72.6 (1)	213.2 (0)	76.3 (1)	75.6 (1)	76.4 (1)	213.4 (0)	213.2 (0)
C-4	135.5 (0)	136.8 (0)	35.8 (1)	34.1 (1)	47.8 (1)	39.8 (1)	35.2 (1)	39.8 (1)	48.1 (1)	47.8 (1)
C-5	146.4 (1)	125.7 (1)	31.9 (2)	31.8 (2)	39.7 (2)	38.5 (2)	39.7 (2)	38.6 (2)	42.0 (2)	35.2 (2)
C-6	45.3 (1)	43.6 (1)	39.0 (1)	38.7 (1)	44.3 (1)	45.2 (1)	44.3 (1)	44.7 (1)	44.5 (1)	44.3 (1)
C-7	45.1 (1)	45.4 (1)	47.8 (1)	47.5 (1)	47.5 (1)	47.8 (1)	47.8 (1)	52.7 (1)	52.7 (1)	42.3 (1)
C-8	26.3 (2)	26.4 (2)	26.3 (2)	26.1 (2)	25.8 (2)	26.1 (2)	26.2 (2)	33.7 (2)	30.7 (2)	26.2 (2)
C-9	35.5 (2)	35.9 (2)	36.4 (2)	35.3 (2)	36.1 (2)	36.4 (2)	44.3 (2)	36.2 (2)	36.0 (2)	35.9 (2)
C-10	149.8 (0)	151.6 (0)	153.3 (0)	152.3 (0)	151.1 (0)	152.3 (0)	150.7 (0)	151.5 (0)	150.7 (0)	150.7 (0)
C-11	16.0 (3)	19.3 (3)	18.6 (3)	11.2 (3)	14.3 (3)	18.5 (3)	14.3 (3)	18.7 (3)	14.3 (3)	10.2 (3)
C-12	26.4 (1)	26.3 (1)	26.0 (1)	26.5 (1)	26.7 (1)	26.5 (1)	42.3 (1)	148.0 (0)	74.0 (0)	39.3 (1)
C-13	15.2 (3)	15.0 (3)	15.0 (3)	14.8 (3)	15.0 (3)	15.0 (3)	10.2 (3)	111.2 (2)	23.9 (3)	66.7 (2)
C-14	21.5 (3)	21.4 (3)	21.6 (3)	21.5 (3)	21.5 (3)	21.6 (3)	66.8 (2)	18.2 (3)	32.4 (3)	14.4 (3)
C-15	105.3 (2)	103.7 (2)	103.4 (2)	103.9 (2)	104.6 (2)	104.0 (2)	105.0 (2)	104.6 (2)	104.8 (2)	105.0 (2)

[a] The number in parentheses indicates the number of hydrogens attached to the corresponding carbon and was determined from DEPT experiments.

Hydroxycadin-10(15)-ene (**4**) and an inseparable mixture of 3α-hydroxycadina-4,10(15)-diene (**2**) and (4S)-3β-hydroxycadin-10(15)-ene (**6**) were obtained. Compound **8** was found to be (4S)-3β-hydroxycadina-10(15),12(13)-diene. This structure was arrived at based on changes in the position of the C-7 resonance and the appearance of two new olefinic signals at 111.2 and 148.0 ppm. The HREIMS of the diene had an M^+ peak at m/z 220.1831 ($C_{15}H_{24}O$).

In cadinane **9** the disappearance of a methine signal at 26.4 ppm pointed to the insertion of a hydroxyl function at C-12. The mass spectrum indicated a peak at m/z 218.1667 which was assigned to $[M - 18]^+$. Thus, **9** was determined to be (4S)-12-hydroxycadin-10(15)-en-3-one. The final product, (4S)-13-hydroxycadin-10(15)-en-3-one (**10**), had a molecular formula of $C_{15}H_{24}O_2$ ($[M]^+ = 236.1772$). A methylene bearing oxygen resonating at 66.7 ppm was present in the ^{13}C NMR spectrum. Also evident was a downfield shift at C-12 and the γ shielding effect at C-14.

The bioconversion of the synthetic alcohol **2** was performed to ascertain the effect, on biotransformation, of replacing the carbonyl oxygen at C-2 by a hydroxyl moiety. Three metabolites were isolated, namely cadina-4,10(15)-dien-3-one (**1**), (4S)-cadin-10(15)-en-3-one (**5**) and (4S)-12-hydroxycadin-10(15)-en-3-one (**9**). Veschambre had indicated that acyclic allylic alcohols are first oxidised before conjugate reduction by this fungus (Bostmembrun-Desrut et al., 1985).

2.1. Insecticidal and phytotoxic studies

The insecticidal potential of all the metabolites against *Cylas formicarius elegantulus* was investigated (Table 2). Ketone **5** showed enhanced activity when compared with the naturally occurring compound (**1**). An increase in activity was also noted for compounds **3** and **7** at higher concentrations. It is of interest to note that the stereochemistries at C-3 and at C-4 had a pronounced effect on the insecticidal properties of the sesquiterpenes. There was marked reduction in activity when the C-4 configuration was *R*. Inversion of the stereochemistry of the 3α-hydroxyl also resulted in a decrease in toxicity. This was, however, less pronounced than that seen for modification at C-4. The insecticidal activities of the metabolites were compared with that of farnesyl methyl ether. The latter is a potent growth regulating agent at low doses, but is also capable of inflicting high mortality on adult *Cylas formicarius* at slightly higher concentrations (Mansingh and Steele, 1975; Mansingh and Rawlins, 1977).

The phytogrowth inhibitory activity of a number of the cadinanes was also determined against *Raphanus sativus* L. (radish) seeds (Table 3). All the compounds tested exhibited some level of inhibition, however, cadinanes **1**, **2**, **3** and **4** had IC_{50} values which were less than that for colchicine. The latter is a commercially available phytotoxic agent which is known to suppress mitosis (Farrell and Wilson, 1980) and hence inhibits germination and supresses radicle elongation.

Therefore, the bioconversion of cadina-4,10(15)-

Table 2
Insecticidal activity of cadinanes against adult *Cylas formicarius elegantulus*

Compounds	Dosage per insect/mg	Mortality/%	
		24 h	24 h
	0.09	35	55
1	0.18	55	85
	0.27	75	95
	0.09	0	0
2	0.18	0	0
	0.27	15	45
	0.09	25	35
3	0.18	85	100
	0.27	100	100
	0.09	0	0
4	0.18	0	0
	0.27	15	20
	0.09	40	60
5	0.18	75	85
	0.27	90	100
	0.09	20	30
6	0.18	40	70
	0.27	60	100
	0.09	40	45
7	0.18	70	80
	0.27	85	100
	0.09	0	0
8	0.18	0	5
	0.27	0	15
	0.09	0	15
9	0.18	45	55
	0.27	65	75
	0.09	0	0
10	0.18	0	10
	0.27	15	35
Farnesyl	0.09	35	75
Methyl	0.18	65	95
Ether	0.27	85	100
Control	0.00	0	0

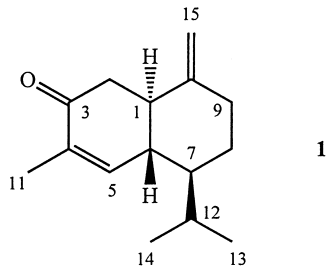
Table 3
Phytogrowth inhibitory activity of cadinanes

Compounds	IC_{50}/μg/ml
1	0.10
2	0.24
3	0.25
4	0.25
5	22.00
9	4.90
10	1.75
Colchicine	0.40

dien-3-one (**1**) by *B. bassiana* ATCC 7159 afforded nine previously unreported metabolites. Three different types of reactions had occurred: reduction, hydroxylation and elimination. A possible sequence of events is as follows: (i) reduction of the 4,5 double bond; (ii) reduction of the carbonyl moiety and (iii) hydroxylation. The synthetic allylic alcohol **2** is probably first converted to the naturally occurring terpene **1** before further biotransformation occurs. The preparation of several novel cadinane analogues, with enhanced insecticidal and phytotoxic potential, has resulted from this study.

3. Experimental

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were taken on a Perkin-Elmer 241 MC polarimeter. IR spectra were recorded on a Perkin-Elmer 735B spectrometer as KBr pellets. ^{1}H and ^{13}C NMR spectra were determined at 200 and 50 MHz, respectively on a Bruker AC200 spectrometer. Samples were run in deuterated chloroform with tetramethylsilane as the internal standard. ^{13}C NMR data is reported in Table 1. HRMS (EI) was done on a Kratos MS50 instrument at an ionising vol-



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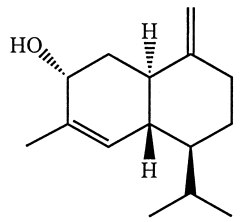

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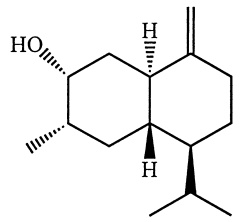

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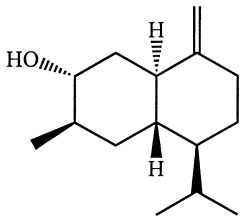

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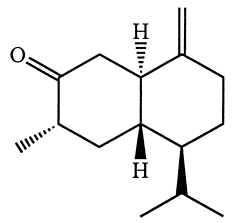

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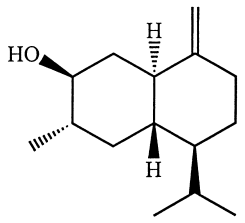

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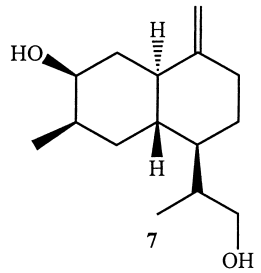

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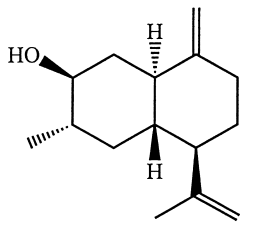

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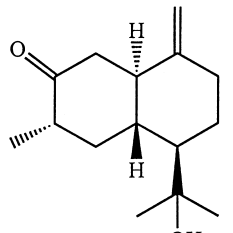

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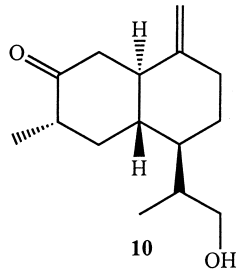

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tage of 70 eV. CIMS (NH_3 reagent gas) were recorded on a VG 7070E spectrometer with an ionising energy of 300 eV. Column chromatography utilised silica gel, 230–400 mesh, and $AgNO_3$–silica gel (1:9). Thin layer chromatography plates were visualised under ultraviolet light and were sprayed with dodecaphosphomolybdic acid/ceric sulfate or ammonium molybdate-sulfuric acid spray before heating. Cadina-4,10(15)-dien-3-one (**1**) was obtained from *Hyptis verticillata* (Jacq.) (12 kg) in an overall yield of 0.05% (Porter et al., 1995). *Beauveria bassiana* ATCC 7159 was obtained from the American Type Culture Collection, Rockville, MD, USA.

3.1. 3α-Hydroxycadina-4,10(15)-diene (2)

To cadina-4,10(15)-dien-3-one (**1**) (2.00 g, 9.13 mmol) in THF (50 ml) was added lanthanum chloride (2.22 g, 9.13 mmol) and the suspension was stirred at room temperature. Lithium aluminium hydride (1.39 g, 36.52 mmol) was added and the mixture was stirred for another 10 min (until effervescence ceased). The mixture was neutralised with dilute hydrochloric acid and extracted with Et_2O (100 ml × 3). The organic layer was dried with magnesium sulfate and concentrated in vacuo to afford a solid. The solid was chromatographed on silica gel and eluted with 3% EtOAc in petrol. This gave 3α-hydroxycadina-4,10(15)-diene (**2**) (1.35 g, 6.14 mmol) (67%). Recrystallisation from petrol–Me_2CO gave needles, mp 115–117°C, $[\alpha]_D$ − 107° ($CHCl_3$: 0.15), IR ν_{max} cm^{-1}: 3402, 3348, 1652, 1044, 896; HRMS (EI) *m/z* (rel. int): 220.1824 $[C_{15}H_{24}O]^+$ (100), 202.1722 $[M - H_2O]^+$, 177.1276 $[M - C_3H_7]^+$ (48.2), 159.1170 $[M - H_2O - C_3H_7]^+$ (64.4), 107.0859 (30.4), 93.0702 (55.5); ^1H NMR: δ 0.74 (3H, *d*, $J = 7.60$ Hz, H-14), 0.90 (3H, *d*, $J = 7.60$ Hz, H-13), 1.78 (3H, *d*, $J = 0.80$ Hz, H-11), 4.25 (1H, *bm*, $w/2 = 19.10$ Hz, H-3), 4.57 (1H, *d*, $J = 0.85$ Hz, H-15), 4.70 (1H, *d*, $J = 0.85$ Hz, H-15), 5.60 (1H, *d*, $J = 0.85$ Hz, H-5).

Also eluting in 3% EtOAc in petrol was (4S)-3α-hydroxycadin-10(15)-ene (**3**) (23 mg), as an oil, IR ν_{max} cm^{-1}: 3432, 2871, 1646; HRMS (EI) *m/z* (rel. int): 222.1981 $[C_{15}H_{26}O]^+$ (24.9), 204.1874 $[M - H_2O]^+$ (21.5), 179.1434 $[M - C_3H_7]^+$ (38.1), 161.1328 $[M - H_2O - C_3H_7]^+$ (100), 133.1015 (33.4), 119.0860 (24.9), 105.0703 (25.6); CIMS *m/z* (rel. int): 240.2 $[M + NH_4]^+$ (73.8), 222.2 $[M]^+$ (34.5), 205.2 $[M - OH]^+$ (100), 161.1 (79), 133.0 (54.3); ^1H NMR: δ 0.69 (3H, *d*, $J = 6.96$ Hz, H-14) 0.90 (3H, *d*, $J = 6.96$ Hz, H-13), 0.98 (3H, *d*, $J = 6.96$ Hz, H-11), 2.02 (1H, *m*, $w/2 = 21.33$ Hz, H-12), 3.92 (1H, *bd*, $J = 2.82$ Hz, H-3), 4.49 (1H, *bs*, H-15), 4.63 (1H, *d*, $J = 1.58$ Hz, H-15).

Further elution with 3% EtOAc in petrol gave (4R)-3α-hydroxycadin-10(15)-ene (**4**) (11 mg) which crystallised from EtOH as amorphous crystals, mp 132–135°C, $[\alpha]_D$ − 13° ($CHCl_3$: 0.05), IR ν_{max} cm^{-1}: 3299, 1642, 1468, 1076, 1060; HRMS (EI) *m/z* (rel. int): 222.1983 $[C_{15}H_{26}O]^+$ (30.8), 204.1872 $[M - H_2O]^+$ (40.0), 179.1431 $[M - C_3H_7]^+$ (23.2), 168.1509 (52.5), 161.1324 $[M - H_2O - C_3H_7]^+$ (88.4), 150.1410 (100); ^1H NMR: δ 0.70 (3H, *d*, $J = 7.00$ Hz, H-13) 0.88 (3H, *d*, $J = 7.00$ Hz, H-14), 0.97 (3H, *d*, $J = 7.30$ Hz, H-11), 3.87 (1H, quintet, $J = 5.10$ Hz, H-3), 4.56 (1H, *d*, $J = 1.30$ Hz, H-15), 4.67 (1H, *d*, $J = 1.60$ Hz, H-15).

3.2. Feeding protocol

The fungus was grown on potato dextrose agar slants. The liquid growth medium contained glucose (10 g/l) and corn steep solids (10 g/l) and was adjusted to pH 4.60 using 2 M aq. sodium hydroxide. One 14-day-old slant was used to inoculate four 500 ml conical flasks each containing 125 ml liquid medium. The flasks were incubated at 200 rpm at 27°. A solution 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 after inoculation, respectively. The fermentation was allowed to proceed for either 10 or 14 days. The pH was measured and the mycelium was filtered from the broth. Broth extraction utilised EtOAc (2 × 750 ml). The mycelium was homogenised in EtOAc. The extracts were dried with sodium sulfate, concentrated in vacuo, and analysed by thin layer chromatography.

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

Cadina-4,10(15)-dien-3-one (**1**) (200 mg) was fed to *B. bassiana* in 20 flasks as outlined above. After 8.5 days the fungus was harvested to give a broth extract of 0.1277 g and mycelial extract of 0.3109 g. The pH of the medium at the end of fermentation was 8.4. Analysis of both extracts by TLC indicated the presence of biotransformed compounds. The mycelial extract was subjected to column chromatography using increasing concentrations of EtOAc in petrol. The fraction eluting in 2.5% EtOAc in petrol afforded (4S)-cadin-10(15)-en-3-one (**5**) (51.6 mg), as a pale brown oil, IR ν_{max} cm^{-1}: 1715, 1640, 1621; HRMS (EI) *m/z* (rel. int): 220.1826 $[C_{15}H_{24}O]^+$ (100), 177.1276 $[M - C_3H_7]^+$ (85.4), 107.0860 (39.3), 93.0701 (49.8), 79.0549 (32.3); CIMS *m/z* (rel. int): 238.2 $[M + NH_4]^+$ (100), 221.1 $[M + H]^+$ (67), 35.0 (81); ^1H NMR: δ 0.77 (3H, *d*, $J = 6.95$ Hz, H-14), 0.94 (3H, *d*, $J = 6.96$ Hz, H-13), 1.04 (3H, *d*, $J = 6.33$ Hz, H-11), 2.03 (1H, *m*, $w/2 = 22.45$ Hz, H-12), 2.39 (1H, *m*, $w/2 = 29.93$ Hz, H-4), 4.71 (1H, *d*, $J = 1.61$ Hz, H-15).

Further elution with 5% EtOAc in petrol afforded (4S)-3α-hydroxycadin-10(15)-ene (**3**) (8.2 mg). Upon further elution of the column with 5% EtOAc in petrol (4S)-3β-hydroxycadin-10(15)-ene (**6**) (6.6 mg) was

obtained as a gum, IR ν_{max} cm^{-1}: 3482, 3419, 1643, 1618; HRMS (EI) *m/z* (rel. int): 222.1982 $[C_{15}H_{26}O]^+$ (28.8), 204.1871 $[M - H_2O]^+$ (35.8), 179.1433 $[M - C_3H_7]^+$ (26.3), 168.1505 (41.2), 161.1325 $[M - H_2O - C_3H_7]^+$ (93.1), 150.1408 (100), 93.0701 (36.1); CIMS *m/z* (rel. int): 240.2 $[M + NH_4]^+$ (50.7), 205.2 $[M - OH]^+$ (100), 35.1 (89); ^1H NMR: δ 0.70 (3H, *d*, $J =$ 6.96 Hz, H-14), 0.90 (3H, *d*, $J = 6.96$ Hz, H-13), 1.04 (3H, *d*, $J = 6.33$ Hz, H-11), 2.01 (1H, *m*, $w/2 = 32.88$, H-12,), 2.36 (2H, *m*, $w/2 = 18.30$ Hz, H-12, 4), 3.2 (1H, *tt*, $J = 6.10$, 8.98 Hz, H-3), 4.55 (1H, *bd*, $J = 1.27$ Hz, H-15), 4.65 (1H, *bd*, $J = 1.58$ Hz, H-15).

Purification of the broth extract afforded a mixture of **1** and **5** (21.1 mg) as well as several other compounds. However, only one compound was present in sufficient yield for characterisation. (4R)-3β,14-Dihydroxycadin-10(15)-ene (**7**) (7.7 mg) eluted from the column in 15% EtOAc in petrol as a gum, IR ν_{max} cm^{-1}: 3421, 3480, 1637, 1617; HRMS (EI): *m/z* (rel. int): 236.1769 $[M - 2]^+$ (28.7), 220.1826 $[M - 18]^+$ (13.2), 179.1423 $[M - C_3H_7O]^+$ (21.1), 177.1275 (100.0), 161.1334 $[M - H_2O - C_3H_7O]^+$ (11.7); ^1H NMR: δ 0.81 (3H, *d*, $J = 6.96$ Hz, H-13), 1.05 (3H, *d*, $J = 6.33$ Hz, H-11), 3.2 (1H, *dt*, $J = 6.12$, 10.20 Hz), 3.55 (2H, *d*, $J = 1.26$ Hz, H-14), 4.49 (1H, *bd*, $J = 1.27$ Hz, H-15), 4.73 (1H, *bd*, $J = 1.59$ Hz, H-15).

A scaled up fermentation of the cadinane (**1**) was performed using twenty 500 ml flasks each containing 250 ml medium. Ten 14-day-old slants were used to inoculate the flasks. The flasks were shaken at 200 rpm. The substrate (1 g) was fed as previously described and incubation was continued for 14 days. This transformation experiment yielded a broth extract of mass 0.3583 g and mycelial extract of mass 0.8728 g on harvest. Column chromatography on the broth extract using increasing concentrations of EtOAc in petrol gave (4S)-cadin-10(15)-en-3-one (**5**) (52.9 mg). The mixture which eluted in 2.5 and 4% EtOAc in petrol (26.2 mg) was further purified via argentation column chromatography. Elution of the column with 30% EtOAc in petrol afforded (4S)-3β-hydroxycadina-10(15),12(13)-diene (**8**) (2.2 mg), which crystallised from EtOH as amorphous crystals, mp 79–83°C, $[\alpha]_D + 121°$ ($CHCl_3$: 0.015), IR ν_{max} cm^{-1}: 3480, 3417, 1637, 1617; HRMS (EI) *m/z* (rel. int): 220.1831 $[C_{15}H_{26}O]^+$ (72.9), 202.1721 $[M - H_2O]^+$ (61.9), 161.1319 $[M - H_2O - C_3H_5]^+$ (22.0), 159.1172 (100), 119.0856 (58.8), 107.0859 (89.0), 105.0702 (63.7); CIMS *m/z* (rel. int): 238.2 $[M + NH_4]$ (16.0), 203.1 $[M - 17]^+$ (40.7), 35.0 (100); ^1H NMR: δ 1.01 (3H, *d*, $J = 6.33$ Hz, H-11), 1.61 (3H, *s*, H-14), 2.38 (1H, *m*, $w/2 = 12.49$Hz, H-3), 3.2 (1H, *dt*, $J = 6.12$, 10.20 Hz, H-3), 4.59 (1H, *d*, $J = 1.58$ Hz, H-5), 4.70 (3H, *m*, $w/2 = 7.59$ Hz, H-13,15).

The fraction which eluted in 8% and 15% EtOAc in petrol (48.8 mg) was further purified on an argentation column. (4S)-12-Hydroxycadina-10(15)-en-3-one (**9**) (30.6 mg) was obtained. This crystallised from EtOH as plates, mp 58–61°C, $[\alpha]_D - 101°$ ($CHCl_3$: 0.082), IR ν_{max} cm^{-1}: 3540, 3412, 1696, 1647; HRMS (EI) *m/z* (rel. int): 218.1667 $[C_{15}H_{24}O_2 - H_2O]^+$ (49.7), 178.13568 (20.7), 160.12495 (30.3), 145.10140 (23.5), 59.04952 (100); CIMS *m/z* (rel. int): 254.2 $[M + NH_4]^+$ (100), 237.1 $[M + H]^+$ (39.3), 35.0 (44.0); ^1H NMR: δ 1.00 (3H, *d*, $J = 6.32$ Hz, H-11), 1.18 (3H, *s*, H-14), 1.24 (3H, *s*, H-13), 2.02 (H, *s*, H-7), 2.91 (1H, *m*, $w/2 = 13.44$ Hz, H-3), 4.47 (1H, *s*, H-15), 4.69 (1H, *d*, $J = 1.58$ Hz, H-15).

A second fraction which eluted with 15% EtOAc in petrol (22.5 mg) was re-purified on an argentation column. Elution with 17.5% EtOAc in petrol afforded (4S)-13-hydroxycadin-10(15)-en-3-one (**10**) (7 mg) as a gum, IR ν_{max} cm^{-1}: 3444, 1716, 1652, 1560; HRMS (EI) *m/z* (rel. int): 236.1772 $[C_{15}H_{24}O_2]^+$ (43.6), 218.1670 $[M - H_2O]^+$ (30.6), 177.1277 $[M - C_3H_7O]^+$ (100), 107.0860 (39.5), 93.0703 (43.8), 55.0547 (37.1); CIMS *m/z* (rel. int): 254.1 $[M + NH_4]^+$ (98.8), 237.2 $[M + H]^+$ (35.6), 35.0 (100); ^1H NMR: δ 0.91 (3H, *d*, $J = 7.30$ Hz, H-14), 1.02 (3H, *d*, $J = 6.60$ Hz, H-14), 3.50 (2H, *dd*, $J = 1.50$, 7.30 Hz, H-13), 4.48 (1H, *d*, $J = 1.70$ Hz, H-15), 4.77 (1H, *d*, $J = 1.70$ Hz, H-15).

Purification of the mycelial extract by column chromatography using increasing concentrations of EtOAc in petrol afforded several compounds. (4S)-Cadin-10(15)-en-3-one (**5**) (198 mg) was eluted from the column with 2% EtOAc in petrol. Also eluting in this solvent system was an 4:1 mixture of **5** and the fed compound (**1**). (4S)-3α-Hydroxycadin-10(15)-ene (**3**) (18.5 mg) eluted from the column in 5% EtOAc in petrol. An inseparable mixture (1:1) of (4S)-3β-hydroxycadin-10(15)-ene (**6**) and 3α-hydroxycadina-4,10(15)-diene (**2**) (140 mg) followed by (4R)-3α-hydroxycadin-10(15)-ene (**4**) (16 mg) were obtained on further elution with the same solvent system.

3.4. Incubation of 3α-Hydroxycadina-4,10(15)-diene (2)

Preparative fermentation of 3α-hydroxycadina-4,10(15)-diene (**2**) (1.0 g) yielded, at harvest, a combined broth and mycelial extract of 1.3594 g. The extract was chromatographed to give a 1:1 mixture of cadina-4,10(15)-dien-3-one (**1**) and cadin-10(15)-en-3-one (**5**) (36.4 mg) on elution with 3% EtOAc in petrol. Percolation with 5% EtOAc in petrol gave the fed compound (**2**) (256 mg).

The fraction which eluted with 20% EtOAc in petrol contained 12-hydroxycadin-10(15)-en-3-one (**9**) and an impurity (40 mg). The impurity was removed by acetylation followed by chromatography to give **9** (20 mg).

3.5. Insecticidal assay

Two-week-old adult *Cylas formicarius elegantulus* weighing 45 ± 0.8 mg each were used for bioassay. Insects were cultured on sweet potato tubers (Ipomoea sp.) in the laboratory at 25 ± 2°C and 65–68% relative humidity (RH). A 4.5% (wt/vol, 11.25 mg in 0.25 ml Me_2CO) stock solution was prepared for all the compounds. From the above 2.0 μl (0.09 mg), 4.0 μl (0.18 mg) and 6.0 μl (0.27 mg) aliquots were topically applied to 20 adult *C. formicarius* in two replicates of 10 each using a Hamilton microapplicator. Twenty insects which were treated with 6.0 μl of Me_2CO served as the controls. The number of dead insects was recorded at 24 and 48 h after treatment.

3.6. Phytotoxic assay

Phytotoxic studies were carried out on *Raphanus sativus* L. var. Scarlet Globe (radish) seeds. The following concentrations: 50, 5.0, 0.5 and 0.05 ppm (μg/l) were prepared from the compounds. Samples were prepared separately by dissolving them in Me_2CO (1.0 ml) before diluting with a mixture of Triton X-100 (0.05 g/l):Me_2CO:water (1:10:89 by volume) which served as the control solvent. Twenty seeds in two replicates of 10 each were placed on two sheets of No. 1 Whatman filter paper in petri dishes of diameter 5.0 cm. The filter papers were soaked with 2.0 ml of each concentration of the different compounds before the seeds were added. The filter papers were subsequently moistened with 1.0 ml of each of the respective test concentrations at 12 h intervals. The number of seeds germinating (i.e. having radicles length greater than 1.5 mm), and their lengths were recorded at 48 and 72 h. The percentage inhibition of radicular elongation was calculated as shown below:

$$\%\ \text{inhibition of radicular growth} = \frac{\text{control radicle length} - \text{treated radicle length}}{\text{control radicle length}} \times 100\%$$

From the inhibition data generated, the IC_{50} (concentration required for inhibiting the growth of the radicle by 50%) was calculated by Probit analysis (Finney, 1971).

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