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Stemodane and stemarane diterpenoid hydroxylation by *Mucor plumbeus* and *Whetzelinia sclerotiorum*

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Dedicated to the memory of Professor Herbert L. Holland (Brock University) for his contribution to Bio-organic Chemistry for over 30 years.

Abstract

Incubation of stemodin (**1**) with *Mucor plumbeus* ATCC 4740 resulted in the formation of 2α,6β,13-trihydroxystemodane (**2**), 2α,3β,13-trihydroxystemodane (**3**), 2α,11β,13-trihydroxystemodane (**4**) and 2α,13,14-trihydroxystemodane (**5**), while stemodinone (**7**) afforded 6α,13-dihydroxystemodan-2-one (**8**) and 6α,12α,13-trihydroxystemodan-2-one (**9**). Metabolites obtained from the bioconversion of stemarin (**11**) were 8,13,19-trihydroxystemarane (**12**) and 2α,13,19-trihydroxystemarane (**13**). 19-*N*,*N*-Dimethylcarbamoxy-13-hydroxystemarane (**14**) was not transformed by the fungus. Stemodin (**1**) was incubated with *Whetzelinia sclerotiorum* ATCC 18687 to produce 2α,7β,13-trihydroxystemodane (**6**) and 2α,11β,13-trihydroxystemodane (**4**). Stemodinone (**7**) was converted to 7β,13-dihydroxystemodan-2-one (**10**). Compounds **2**, **4**, **9**, **10**, **12** and **13** have not been previously reported.

Keywords: *Mucor plumbeus* ATCC 4740; *Whetzelinia sclerotiorum* ATCC 18687; *Sclerotinia sclerotiorum*; Biotransformation; Stemodane; Stemarane; Diterpene; *Stemodia maritima*; Hydroxylation

1. Introduction

As a part of our programme to produce analogues of biologically active natural products using fungi (Buchanan and Reese, 2000; Collins et al., 2001), three diterpenes with stemodane and stemarane skeleta and a synthetic analogue were incubated with *Mucor plumbeus* (formerly *M. spinosus*) ATCC 4740. The fungus has been used previously to transform terpenes possessing stemodane (Fraga et al., 2004), labdane (Aranda et al., 1991; Azerad, 2000), cedrane (Fraga et al., 1996), and aromadendrane (Guillermo et al., 1997) skeleta. Other terpenoids have also been transformed (Aranda et al., 1992). Furthermore the potential of the ascomycete *Whetzelinia* (formerly *Sclerotinia*) *sclerotiorum* ATCC 18687 for bioconversion was examined for the first time.

Diterpenes possessing mild antiviral and cytotoxic properties have been isolated from *Stemodia maritima* (Hufford et al., 1991). Three of these are stemodin (**1**), stemodinone (**7**) and stemarin (**11**). The bioconversion of compounds **1** and **7** (Azerad, 2000) using *Cunninghamella echinulata* (Badria and Hufford, 1991; Hufford et al., 1991), *Polyangium cellulosum* (Badria and Hufford, 1991), *Rhizopus arrhizus* (Hufford et al., 1991; Martin et al., 2004), *Cephalosporium aphidicola* (Hanson et al., 1994), *Beauveria bassiana* (Buchanan and Reese, 2001) and *Aspergillus niger* (Chen and Reese, 2002) have been reported.

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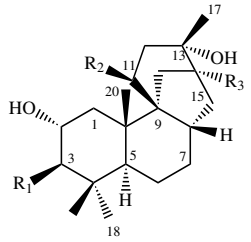
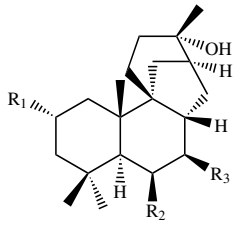
2. Results and discussion

2.1. M. plumbeus

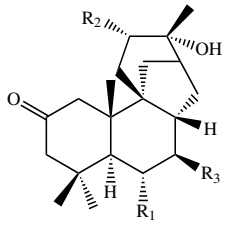
The incubation of **1** with this fungus afforded four metabolites (**2**–**5**). HREIMS analysis of the diacetate (**2a**) of **2** suggested a molecular formula of $C_{24}H_{38}O_5$. ^{13}C NMR data revealed a new methine signal at δ 81.3. The C-6α proton (δ 4.62) showed ^1H–^1H COSY correlations with the C-7 protons. T-ROESY enhancements with H-5 (δ 1.32), H-7 (δ 1.77), H-15α (δ 1.61) and H-16α (δ 1.51) established the equatorial orientation of H-6 in **2a**. Thus **2** was shown to be 2α,6β,13-trihydroxystemodane, a new compound. Diterpene **3** (maristeminol) was isolated previously as a natural product from *S. maritima* (Hufford et al., 1992). The appearance of a new methine at 70.5 ppm in the ^{13}C NMR spectrum suggested that **4** was a hydroxylation product. This was supported by HREIMS data which confirmed a molecular formula of $C_{20}H_{34}O_3$. It was determined that the hydroxyl group was borne by C-11. Even though C-11 and C-12 protons exhibited HMBC correlations to the same carbons, H-17 showed HMBC correlations with the carbon at δ 44.8 (C-12) and not with that at 70.5 ppm (C-11). The stereochemistry of the hydroxyl group was assigned as β since H-11α showed strong NOE cross-peaks with H-20 and H-1β. The latter coupling would not be expected with an 11 proton. Metabolite **4** is thus the hitherto unreported 2α, 11β,13-trihydroxystemodane. Analogue **5** (2α,13,14-trihydroxystemodane) has been reported from a biotransformation experiment using *P. cellulosum* (Badria and Hufford, 1991).



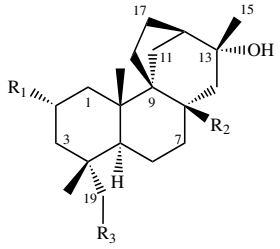
1 R_1=R_2=R_3=H
3 R_1=OH, R_2=R_3=H
4 R_1=R_3=H, R_2=OH
5 R_1=R_2=H, R_3=OH



2 R_1=R_2=OH, R_3=H
2a R_1=R_2=OAc, R_3=H
6 R_1=R_3=OH, R_2=H
6a R_1=R_3=OAc, R_2=H



7 R_1=R_2=R_3=H
8 R_1=OH, R_2=R_3=H
9 R_1=R_2=OH, R_3=H
10 R_1=R_2=H, R_3=OH



11 R_1= R_2=H, R_3=OH
12 R_1=H, R_2=R_3=OH
13 R_1=R_3=OH, R_2=H
14 R_1= R_2=H, R_3=OC(O)N$(CH_3)_2$

When ketone **7** was fed to the growing fungus two metabolites **8** and **9** were isolated. Monohydroxylation at C-6 afforded 6α,13-dihydroxystemodan-2-one (**8**) (Martin et al., 2004). Two new methine signals at δ 69.1 and 70.6 were observed in the ^{13}C NMR spectrum of **9**. The data suggested that the metabolite was formed by hydroxylation at both C-6 and -12. The stereochemistry of H-6 was deduced to be β since key NOE interactions were seen with H-19, -20 and -7β (but not with H-5). The position and stereochemistry of the C-12 hydroxyl group was determined as had been for **4**, i.e. based on HMBC and NOE data. Metabolite **9** was therefore 6α,12α,13-trihydroxystemodan-2-one.

Incubation of stemarin (**11**) with the fungus yielded two new metabolites, **12** and **13**, which were more polar than the starting material. The HRMS data for **12** indicated a molecular formula of $C_{20}H_{34}O_3$. Examination of the ^{13}C and 2D NMR spectra indicated that hydroxylation had taken place at one of the methine carbons. This was evidenced by a new peak at δ 76.7, which was assigned to a non-protonated carbon. β and γ shift effects due to hydroxyl substitution established that functionalisation was at C-8 (rather than C-5 or C-12). Thus the metabolite was 8,13,19-trihydroxystemarane. HRMS analysis of **13** showed that it possessed the same molecular formula as **12**. However, inspection of the ^{13}C NMR data showed the disappearance of the C-2 signal (18.3 ppm) from **11** and the appearance of a new peak at 65.7 ppm. H-2β showed NOE interactions with H-1β, -3β, -18 and -20 only. **13** was deduced to be 2α,13,19-trihydroxystemarane.

In an effort to improve the docking potential of the substrate the dimethylcarbamate derivative (**14**) of stemarin (Vigne et al., 1986, 1991) was prepared by treatment of **11** with dimethylcarbamyl chloride (Jager et al., 1968; Agarwal and Khorana, 1972). HREIMS analysis of **14** indicated a molecular formula of $C_{23}H_{39}NO_3$ (M^+ = 377.2930). After an incubation period of two weeks **14** had not been metabolised by the fungus. This was surprising since the carbamate group was expected be a better binding group than the alcohol. It is possible that the lack of hydroxylation may be due to steric, rather than electronic, factors.

2.2. Whetzelinia sclerotiorum

This fungus has not been exploited previously as an agent for biocatalysis, however, fermentation conditions

for its growth have been reported (Bhutani and Thakur, 1991). Unfortunately, the culture medium described (designated for our purpose as medium A) promoted the production of a polyglucan by the organism (Ohno et al., 1988; Keats et al., 1998) which was excreted into the broth. Extraction of the fermentation beer by EtOAc resulted in the formation of an emulsion separable only by freezing out of the aqueous layer. This made recovery of transformed metabolites extremely poor. Since the biosynthesis of this polysaccharide should depend on glucose availability, it was decided to replace the latter by cellulose for the most part (medium B). The organism has been reported to produce cellulase (Lumsden, 1969). Happily the formation of polyglucan in this reformulated medium was satisfactorily minimised.

Incubation of stemodin (**1**) afforded two metabolites **4** and **6**. The latter (2α,7β,13-trihydroxystemodane) has been isolated previously (Badria and Hufford, 1991; Hanson et al., 1994). This compound was characterised as its 2,7-diacetate (**6a**).

Incubation of stemodinone (**7**) yielded a new metabolite (**10**). Examination of the spectral data showed some similarities with those of **6**. Loss of the methylene at δ 36.4 (C-7) and the appearance of a methine at δ 78.5 were observed. The coupling constants suggested that H-7 had an axial orientation. This was confirmed from NOE interactions of H-7α with H-15α, -5 and -6α. ^{1}H–^{1}H COSY correlations were in agreement with this assignment. Thus **10** was found to be 7β,13-dihydroxystemodan-2-one. Stemarin (**11**) was not incubated with *W. sclerotiorum* because the quantities of this terpene isolated from the plant were insufficient.

In summary three substrates were fed to two fungi to afford 10 metabolites, six of which were new. A new growth medium, which minimised the production of polyglucan, was formulated for *W. sclerotiorum*. Bioconversion of substrates by this fungus was observed for the first time.

3. Experimental

3.1. General experimental

Melting points were recorded on a Thomas Hoover melting point apparatus and are uncorrected. IR spectra were recorded as KBr disks using a Perkin–Elmer 735B infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded using Bruker AC200 and Varian Unity 500 NMR spectrometers. NMR samples were prepared in $CDCl_3$ containing tetramethylsilane as the internal standard. ^{13}C NMR assignments are reported in Table 1. Optical rotations were acquired on a Perkin–Elmer 241 MC polarimeter. HRMS (EI) was done on a Kratos MS50 instrument at an ionising voltage of 70 eV. ESMS analysis was performed on Agilent Technologies 1100MSD or Micromass Zabspec-oaTOF spectrometers. Column chromatography utilised silica gel (230–400 mesh) for purifications. Thin layer chromatography plates were visualised by spraying with ammonium molybdate–sulfuric acid spray and heating until the colours developed. Stemodin (**1**) and stemarin (**11**) were obtained from *Stemodia maritima* in overall yields of 0.07% and 0.02%, respectively. *M. plumbeus* ATCC 4740 and *W. sclerotiorum* ATCC 18687 were obtained from the American Type Culture Collection, Rockville, MD, USA. Petrol refers to the petroleum fraction boiling at 60–80 °C.

Table 1
^{13}C NMR chemical shift assignments for biotransformed metabolites or their derivatives

Carbon	**2a**	**4**	**6a**	**9**	**10**	**12**	**13**
1	41.7	46.2	41.6	51.3	51.7	31.4	41.7
2	68.8	65.0	68.8	212.0	214.4	18.3	65.7
3	46.3	50.5	46.2	56.8	55.9	35.8	44.8
4	34.6	34.6	34.5	38.9	39.3	38.5	39.5
5	43.9	46.5	42.8	52.3	45.1	42.5	40.7
6	81.3	21.8	27.3	69.1	31.7	32.9	22.2
7	27.4	35.3	81.3	45.6	78.5	36.5	30.4
8	42.9	31.7	43.9	37.0	46.8	76.7	38.3
9	52.0	55.3	51.9	50.9	51.9	56.3	51.6
10	39.8	41.2	39.7	45.0	45.1	39.8	40.5
11	27.9	70.5	27.8	37.6	28.4	18.8	27.5
12	32.7	44.8	32.6	70.6	32.5	47.5	48.1
13	72.2	73.4	72.2	72.4	72.0	74.4	73.9
14	46.1	44.8	46.0	45.2	46.1	50.9	39.5
15	35.7	38.0	35.6	37.5	36.6	29.4	29.5
16	31.5	29.2	31.4	29.7	32.1	27.7	29.6
17	28.2	28.1	28.1	24.1	27.8	24.3	26.7
18	34.4	34.9	34.4	37.3	34.4	20.0	18.8
19	23.5	23.8	21.5	25.1	24.1	72.0	70.9
20	19.6	22.2	19.5	19.7	19.2	18.0	18.0
CO	170.6		170.7				
CH_3	21.4		21.4				
CO	171.0		171.1				
CH_3	21.5		23.5				

3.2. M. plumbeus

3.2.1. General fermentation protocol

The fungus was grown on potato dextrose agar slants. Two 14 day old slants were used to inoculate twenty 500 ml Erlenmeyer flasks each containing 125 ml culture medium. This medium was comprised of, per litre, glucose (30 g), corn steep solids (5 g), $NaNO_3$ (2 g), KCl (0.5 g), $MgSO_4 \cdot 7H_2O$ (0.5 g) and $FeSO_4 \cdot 7H_2O$ (0.02 g). The flasks were shaken at 250 rpm. Seventy two hours after inoculation substrates, dissolved in EtOH, were fed to the growing fungus. The cultures were left for an additional 10 days after which the mycelia and broth were separated, and then each was extracted with EtOAc.

3.2.2. Incubation of stemodin (*1*)

Stemodin (500 mg) dissolved in EtOH (10 ml) was fed to the growing fungus. Extraction of the broth and mycelia and removal of the solvent afforded a gum (707 mg) which was chromatographed. Elution with 30% EtOAc in petrol afforded unchanged stemodin (131 mg). Further elution yielded 2α,6β,13-trihydroxystemodane (**2**) (22 mg), characterised as the 2,6-diacetate (**2a**) which resisted crystallisation, $[\alpha]_D - 4.3°$ (*c* 5.8, $CHCl_3$); ESMS: *m/z* (rel. int.) 429.2612 $[M + Na]^+$ (100); IR: ν_{max} cm^{-1} 3452, 2940, 1734, 1684; ^1H NMR: δ 0.98 (3H, *s*, H-18), 0.97 (3H, *s*, H-19), 1.09 (3H, *s*, H-20), 1.13 (3H, *s*, H-17), 2.01 (3H, *s*, CH_3CO_2), 2.06 (3H, *s*, CH_3CO_2), 3.64 (1H, *s*, OH), 4.62 (1H, *m*, $w/2 = 23.7$ Hz, H-6α), 4.91 (1H, *tt*, $J = 3.8$, 11.9 Hz, H-2β).

Further elution gave 2α,3β,13-trihydroxystemodane (**3**) (20 mg) which crystallised from acetone as needles, m.p. 182–184 °C, $[\alpha]_D + 17.1°$ (*c* 3.6, $CHCl_3$) [lit. m.p. 180–182 °C, $[\alpha]_D + 17.5°$ (*c* 1.0, MeOH) (Hufford et al., 1992)]; the spectral data of which was identical to that in the literature. Further elution afforded 2α,11β,13-trihydroxystemodane (**4**) (23 mg) which did not crystallise, $[\alpha]_D - 48.2°$ (*c* 1.7, $CHCl_3$); EIMS: *m/z* 304.2402 (**7**) $([M - H_2O]^+)$; ESMS: *m/z* (rel. int.) 345.2400 $[M + Na]^+$ (100); IR: ν_{max} cm^{-1} 3440, 2940, 1648; ^1H NMR: δ 0.94 (3H, *s*, H-19), 0.97 (3H, *s*, H-18), 1.16 (3H, *s*, H-17), 1.25 (3H, *s*, H-20), 3.86 (1H, *m*, $w/2 = 24$ Hz, H-2β), 4.21 (1H, *d*, $J = 10.4$ Hz, H-11α).

Further elution produced 2α,13,14-trihydroxystemodane (**5**) (9 mg) which did not crystallise, $[\alpha]_D + 33.3°$ (*c* 2.4, $CHCl_3$) [lit. m.p. 190–191 °C (Badria and Hufford, 1991)]; the spectral data of which was identical to that in the literature.

3.2.3. Incubation of stemodinone (*7*)

Stemodinone (1.0 g) was dissolved in EtOH (20 ml) and fed to the fungus. Work up afforded a gum (2.8 g) which was purified by chromatography. Elution with 20% EtOAc in petrol gave stemodinone (480 mg).

Further elution yielded 6α,13-dihydroxystemodan-2-one (**8**) (125 mg) which crystallised as needles from acetone, m.p. 191–193 °C, $[\alpha]_D + 55.6°$ (*c* 4.5, $CHCl_3$), the spectral data of which was identical to that in the literature (Martin et al., 2004).

Further elution gave 6α,12α,13-trihydroxystemodan-2-one (**9**) (6 mg) which did not crystallise, $[\alpha]_D + 28.5°$ (*c* 0.7, $CHCl_3$); ESMS: *m/z* (rel. int.) 359.2193 $[M + Na]^+$ (100); IR: ν_{max} cm^{-1} 3402, 2972, 2933, 1705; ^1H NMR: δ 1.03 (3H, *s*, H-20), 1.20 (3H, *s*, H-19), 1.22 (3H, *s*, H-17), 1.32 (3H, *s*, H-18), 3.50 (1H, *q*, $J = 6.6$ Hz, H-12β), 3.88 (1H, *dt*, $J = 4.7$, 9.8 Hz, H-6β).

3.2.4. Incubation of stemarin (*11*)

Stemarin (1.0 g), dissolved in EtOH (20 ml), was fed to the fungus. The fermentation conditions were the same except that the broth was acidified to pH 2 using 3 M HCl before extraction. The resulting gum (1.7 g) was chromatographed. Elution with 5% acetone in dichloromethane afforded untransformed stemarin (270 mg).

Further elution with 25% acetone in dichloromethane gave 8,13,19-trihydroxystemarane (**12**) (11 mg) which did not crystallise, $[\alpha]_D + 10.8°$ (*c* 8.1, $CHCl_3$); EIMS: *m/z* (rel. int.) 320.2529 (0.8) ($[M^+]$), 304.2411 (67), 291.2326 (8), 273.2226 (100); IR: ν_{max} cm^{-1} 3388, 2940, 2872, 2366; ^1H NMR: δ 0.77 (3H, *s*, H-20), 1.16 (3H, *s*, H-18), 1.90 (3H, *s*, H-15), 3.03 (1H, d, $J = 11.3$ Hz, H-19), 3.48 (1H, *d*, $J = 11.3$ Hz, H-19).

Further elution afforded 2α,13,19-trihydroxystemarane (**13**) (547 mg) which resisted crystallisation, $[\alpha]_D + 13.0°$ (*c* 20.5, $CHCl_3$); EIMS: *m/z* (rel. int.) 322.2519 (2) ($[M^+]$), 291.2338 (10), 286.2310 (10), 273.2229 (100); IR: ν_{max} cm^{-1} 3484, 2944, 2350; ^1H NMR: δ 0.78 (3H, *s*, H-18), 1.00 (3H, *s*, H-20), 1.15 (3H, *s*, H-15), 3.00 (1H, *d*, $J = 11.7$ Hz, H-19), 3.44 (1H, *d*, $J = 11.7$ Hz, H-19), 4.00 (1H, *m*, $w/2 = 19.6$ Hz, H-2β).

3.2.5. Synthesis of 19-*N*,*N*-dimethylcarbamoxy-13-hydroxystemarane (*14*)

The dimethylcarbamate was prepared as reported previously (Chen and Reese, 2002).

3.3. *W. sclerotiorum*

3.3.1. General fermentation protocol

The microorganism was grown on potato dextrose agar slants at 28 °C for two weeks. Five slants were used to inoculate twenty 500 ml Erlenmeyer flasks each containing 125 ml culture medium. Medium A (Modified Richard's medium) was comprised of, per litre, glucose (40 g), yeast extract (2 g), KNO_3 (10 g), $MgSO_4 \cdot 7H_2O$ (1.5 g), and KH_2PO_4 (2.5 g) (Bhutani and Thakur, 1991). Medium B consisted of, per litre, glucose (0.5 g), cellulose (20 g), yeast extract (0.5 g), KNO_3 (10 g), $MgSO_4 \cdot 7H_2O$ (1.5 g) and KH_2PO_4 (2.5 g). The flasks were shaken at 230 rpm. Seventy two hours after inoculation substrates, dissolved in EtOH, were fed to the growing fungus in portions of 10%, 20%, 30% and 40% at 24, 36, 48 and 60 h, respectively after inoculation. The fermentation was allowed to proceed for a further 10 days after the final feed. Extraction of the broth and mycelia with EtOAc and removal of the solvent afforded gums.

3.3.2. Incubation of stemodin (*1*) (medium B)

Stemodin (**1**) (500 mg) in EtOH (10 ml) was fed to the fungus. Chromatography of the resultant gum (0.46 g) using 40% EtOAc in petrol afforded stemodin (216 mg).

Further elution yielded 2α,7β,13-trihydroxystemodane (**6**) (189 mg). This was characterised as the 2,7-diacetate (**6a**) which resisted crystallisation, $[\alpha]_D + 2.5°$ (*c* 35.5, $CHCl_3$); EIMS: *m/z* (rel. int.) 388.2614 (6) ($[M - H_2O]^+$), 346.2508 (20), 335.2222 (10), 328.2402 (27), 286.2297 (100); IR: ν_{max} cm^{-1} 3509, 2963, 2877, 1733; ^1H NMR: δ 0.97 (3H, *s*, H-18), 0.98 (3H, *s*, H-19), 1.09 (3H, *s*, H-20), 1.13 (3H, *s*, H-17), 2.02 (3H, *s*, CH_3CO_2), 2.06 (3H, *s*, CH_3CO_2), 4.63 (1H, *t*, $J = 10.1$ Hz, H-7α), 4.91 (1H, *m*, $w/2 = 18.0$ Hz, H-2β).

Further elution gave 2α,11β,13-trihydroxystemodane (**4**) (26 mg) which was identified by comparison of its spectral data with that of an authentic sample.

3.3.3. Incubation of stemodinone (7) (medium B)

Stemodinone (500 mg) in EtOH (10 ml) was fed and the resultant gum (1.09 g), after work up, was purified using column chromatography. Elution with 15% acetone in CH_2Cl_2 afforded stemodinone (197 mg).

Further elution gave 7β,13-dihydroxystemodan-2-one (**10**) (108 mg) which crystallised as cubes from acetone, m.p. 106–108 °C, $[\alpha]_D + 14.8°$ (*c* 3.8, acetone/MeOH); EIMS: *m/z* (rel. int.) 320.2343 (34) (M^+), 302.2242 (22) ($[M - H_2O]^+$), 287.2007 (15), 284.2134 (12), 233.9105 (93); IR: ν_{max} cm^{-1} 3398, 2963, 2876, 1694, 1385; ^1H NMR: δ 0.97 (3H, *s*, H-19), 1.00 (3H, *s*, H-20), 1.12 (3H, *s*, H-18), 1.13 (3H, *s*, H-17), 3.41 (1H, *m*, $w/2 = 13.0$ Hz, H-7α).

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References

Agarwal, K.L., Khorana, H.G., 1972. Studies on polynucleotides. CII. The use of aromatic isocyanates for selective blocking of the terminal 3′-hydroxyl group in protected deoxyribooligonucleotides. J. Am. Chem. Soc. 94, 3578–3585.

Aranda, G., Kortbi, M.S., Lallemand, J., Neuman, A., Hammoumi, A., Facon, I., Azerad, R., 1991. Microbial transformation of diterpenes: hydroxylation of sclareol, manool and derivatives by *Mucor plumbeus*. Tetrahedron 47, 8339–8350.

Aranda, G., Facon, I., Lallemand, J., Leclaire, M., Azerad, R., Cortes, M., Lopez, J., Ramirez, H., 1992. Microbial hydroxylation in the drimane series. Tetrahedron Lett. 33, 7845–7848.

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.

Badria, F.A., Hufford, C.D., 1991. Microbial transformations of stemodin, a *Stemodia* diterpene. Phytochemistry 30, 2265–2268.

Bhutani, K.K., Thakur, R.N., 1991. The microbiological transformation of parthenin by *Beauveria bassiana* and *Sporotrichum pulverulentum*. Phytochemistry 30, 3599–3600.

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

Buchanan, G.O., Reese, P.B., 2001. Biotransformation of diterpenes and diterpene derivatives by *Beauveria bassiana* ATCC 7159. Phytochemistry 56, 141–151.

Chen, A.R.M., Reese, P.B., 2002. Biotransformation of terpenes from *Stemodia maritima* by *Aspergillus niger* ATCC 9142. Phytochemistry 59, 57–62.

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.

Fraga, B.M., Guillermo, R., Hanson, J.R., Truneh, A., 1996. Biotransformation of cedrol and related compounds by *Mucor plumbeus*. Phytochemistry 42, 1583–1586.

Fraga, B.M., Guillermo, R., Hernandez, M., Chamy, M.C., Garbarino, J.A., 2004. Biotransformation of two stemodane diterpenes by *Mucor plumbeus*. Tetrahedron 60, 7921–7932.

Guillermo, R., Hanson, J.R., Truneh, A., 1997. Hydroxylation of aromadendrane derivatives by *Mucor plumbeus*. J. Chem. Res. (S), 28–29.

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.

Hufford, C.D., Badria, F.A., Abou-Karram, M., Shier, W.T., Rogers, R.D., 1991. Preparation, characterisation, and antiviral activity of microbial metabolites of stemodin. J. Nat. Prod. 54, 1543–1552.

Hufford, C.D., Oguntimien, B.O., Muhammad, I., 1992. New stemodane diterpenes from *Stemodia maritima*. J. Nat. Prod. 55, 48–52.

Jager, G., Geiger, R., Siedel, W., 1968. Peptidsynthesen mit *O*-carbamoyl-tyrosin-derivaten. Chem. Ber. 101, 2762–2770.

Keats, S.E., Loewus, F.A., Helms, G.L., Zink, D.L., 1998. 5-*O*-(α-D-Galactopyranosyl)-D-glycero-pent-2-enono-1,4-lactone: characterisation in the oxalate-producing fungus, *Sclerotinia sclerotiorum*. Phytochemistry 49, 2397–2402.

Lumsden, R.D., 1969. *Sclerotinia sclerotiorum* infection of bean and the production of cellulase. Phytopathology 59, 653–657.

Martin, G.D.A., Reynolds, W.F., Reese, P.B., 2004. Investigation of the importance of the C-2 oxygen function in the transformation of stemodin analogues by *Rhizopus oryzae* ATCC 11145. Phytochemistry 65, 701–710.

Ohno, N., Kurachi, K., Yadomae, T., 1988. Physicochemical properties and antitumor activities of carboxymethylated derivatives of glucan from *Sclerotinia sclerotiorum*. Chem. Pharm. Bull. 36, 1016–1025.

Vigne, B., Archelas, A., Fourneron, J.D., Furstoss, R., 1986. Microbial transformations. Part 4 (I). Regioselective *para*-hydroxylation of aromatic rings by the fungus *Beauveria sulfurescens*. The metabolism of isopropyl *N*-phenyl carbamate (Prophar®). Tetrahedron 42, 2451–2456.

Vigne, B., Archelas, A., Furstoss, R., 1991. Microbial transformations 18. Regiospecific *para*-hydroxylation of aromatic carbamates mediated by the fungus *Beauveria sulfurescens*. Tetrahedron 47, 1447–1458.