Fungal terpene metabolites: biosynthetic relationships and the control of the phytopathogenic fungus *Botrytis cinerea*

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The structures and biosynthesis of the sesquiterpenoid metabolites of *Botrytis cinerea* and their relationship to the presilphiperfolanes are reviewed. The development of a novel strategy for the control of this phytopathogenic fungus based on analogues of these metabolites is described. There are 75 references.

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1 Introduction

There are a wide variety of structural types of sesquiterpenoids, particularly amongst those that are produced by fungi. Two (at first sight) unrelated families of sesquiterpenoids are the botryanes (botrydial (**1**)) derived from the fungal plant pathogen *Botrytis cinerea*[1] and the presilphiperfolanes (presilphiperfolan-8β-ol (**2**)), which were obtained in the first instance from a plant, the Californian coastal succulent *Eriophyllum staechadifolium*.[2]

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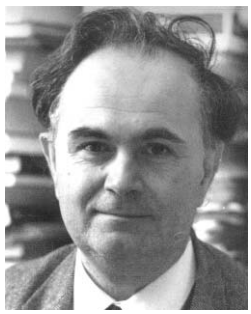
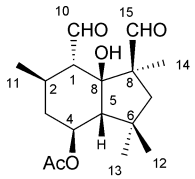
Jim Hanson is a Research Professor at the University of Sussex. His research has been on the biosynthesis and biotransformation of terpenoids and steroids, the chemistry of steroids and in the development of antifungal agents, particularly those that have activity against botrytis.


Isidro Collado

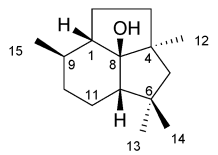

Antonio Macías Sánchez


Jim Hanson

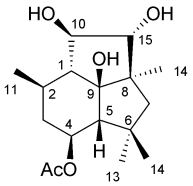
Recent studies have shown that these sequiterpenoids are related. Compound **3** has been isolated from a culture of the fungus *B. cinerea*, and this knowledge has been applied to the development of a means of control of the phytopathogenic fungus *B. cinerea* mediated by secondary metabolites. This relationship and the development of a novel method of control of the fungus forms the subject of this review.



Botrydial **(1)**



Presilphiperfolan-8β-ol **(2)**



4β-Acetoxyprobotryane-9β,10β,15α-triol **(3)**

2 *Botrytis cinerea*

Micro-organisms from the genus *Botrytis* are 'fungi imperfecti' that belong to the order Moniliales of the family Moniliaceae. They are geographically widespread, having been found from cold regions such as Alaska and Canada to sub-tropical regions such as Egypt, and are polyphagous parasites which cause serious economic loss to commercial crops. *B. cinerea* has been identified as a pathogen of more than 235 plant species including grapes, lettuce, tomatoes, tobacco and strawberries, producing a grey powdery mould on the infected crops. Other relatives such as *B. allii*, *B. byssoides* and *B. squamosa* are pathogens on onions, the latter causing stalk breakage. *B. fabae* is a pathogen on beans, causing lesions on the leaves, *B. gladioli* is a pathogen of gladioli and lilies, whilst *B. tulipae* affects tulips and saffron.

B. cinerea is primarily a saprophyte that attacks damaged parts of the plant before spreading to healthy tissue. The most common symptoms of a *Botrytis* infection involves the development of necrotic lesions on the leaves.

The initiation of disease by *Botrytis* species depends on a complex sequence of biological events involving host and environment sensing, chemical and physical interactions between the fungal propagules and the host surface, and microbial interactions on the surface of the host. *Botrytis cinerea* has a broad habitat range, is not restricted with regard to host or tissues, and uses various infection strategies to cope with different conditions. The infection of host plants by *Botrytis* spp. is mediated by numerous extracellular enzymes and metabolites, each of which may play a role in different stages of the infection process. Some cell-wall-degrading enzymes may facilitate the penetration of the host surface, while toxins, oxalate and reactive oxygen species may contribute to killing the host cells.[3]

Once it has penetrated the cuticle, *B. cinerea* kills underlying epidermal cells before they are invaded by hyphae.[4] Invasion of plant tissue by the fungus triggers processes indicative of programmed cell death at a distance from the hyphae, implying that diffusible factors have a direct or indirect phytotoxic activity. The inducing factors may be proteins or low molecular weight compounds secreted by the fungus into its environment. The induction of programmed cell death facilitates *B. cinerea* invasion and may in fact be essential for successful infection.[4–6]

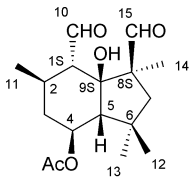
Several secondary metabolites showing phytotoxic and apoptotic[7] activities have been identified in culture filtrates of *B. cinerea*.[8] There is no evidence for the production of host-specific toxins by *B. cinerea*, in accordance with the broad host range of this pathogen. Some interesting features of these toxins, such as their ability to reproduce *in vitro* and *in vivo* the symptoms of a fungal infection by *B. cinerea*, the light-dependence of their phytotoxic effect and their detection in plant tissues infected with isolates of *B. cinerea*,[9] have increased the interest in these compounds and their role in the infection mechanism of this phytopathogen.

3 The metabolites of *Botrytis cinerea*

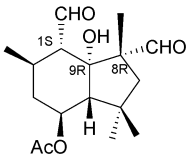
The fungus produces two series of phytotoxic metabolites; a family of polyketide lactones known as the botcinolides[10] and botrylactone[10g,11] and secondly the sesquiterpenoid botryanes. We are concerned with the latter in this review. Botrydial (**1**) and dihydrobotrydial (**4**) were the first metabolites to be isolated from *B. cinerea.* The apparently nonisoprenoid structures of these sesquiterpenoids were elucidated by a combination of chemical, spectroscopic[1] and X-ray crystallographic methods.[12] The absolute configuration was deduced from the negative Cotton effect in the circular dichroism curve of the six-membered lactone ring[13] derived from dihydrobotrydial (**4**). The NMR spectra of these compounds have been assigned and this has facilitated the elucidation of the structures of many more botryane metabolites which have subsequently been isolated from the fungus (compounds **1**–**31**). The formation of the various metabolites is dependent on the culture conditions, such as surface or shake culture and the pH of the fermentation medium.

Compounds differing in the oxidation level of C-10 and C-15 are common. A number of the carboxylic acids were isolated as their methyl esters (compounds **6**, **6a** and **7**)[14] or lactones (compounds **8**[15] and **9**[16]) whilst the 10:15-hemiacetals have also been obtained either free (compounds **4**, **10**[16] and **11**[17]) or as their methyl ethers (compounds **12**,[15] **13a**,[15a] **14**[15a] and **15**[17]).

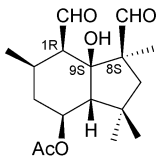
A detailed examination of the fermentation broth led to the isolation of l-epibotrydial (**1b**), 8,9-epibotrydial (**1a**) and 1,8,9-epibotrydial (**1c**).[15b] These compounds might be formed by enolization of the C-10 aldehyde and by retro-aldol fragmentation and reformation of the C-8–C-9 bond. A further series of botryane metabolites are related *via* the dehydration products of botrydial (**1**) (botryendial (**16**), botryenalol (**17**), methyl acetyl botryenaloate (**18**) and botrydienal (**19**)),[16] and 10-oxodihydrobotrydial (**8**) (norbotrydialone acetate (**20**), 10-oxodihydrobotry-1(9),4(5)-diendial (**21**) and 10-oxodehydrodihydrobotrydial (**22**)).[18] The diene may undergo dehydrogenation to form aromatic metabolites (dehydrobotrydienol (**23**), 11-hydroxydehydrobotrydienol (**24**) and 12-hydroxydehydrobotrydienol (**25**)).[17] An electrocyclic ring-opening reaction forms the triene derivatives (secobotrytriendiol (**26**) and secobotrytriene-10,12,15-triol (**27**)).[17,19] Further oxidation products (**28**,**29**) are also observed.[19] These relationships are discussed later.



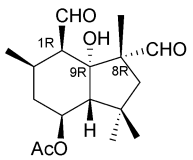
Botrydial **(1)**



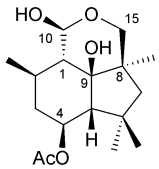
8,9-Epibotrydial **(1a)**



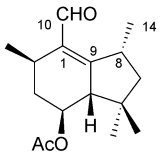
1-Epibotrydial **(1b)**



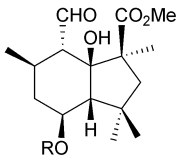
1,8,9-Epibotrydial **(1c)**



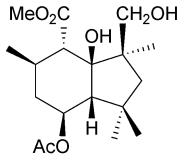
Dihydrobotrydial **(4)**



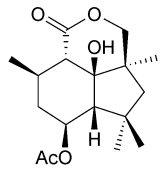
Norbotryal acetate **(5)**



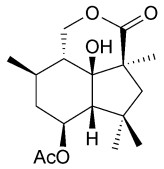
Methyl botryaloate (R = H) **(6)**
Methyl botryaloate acetate (R = Ac) **(6a)**



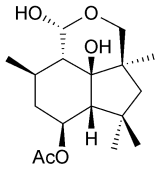
Methyl botryoloate **(7)**



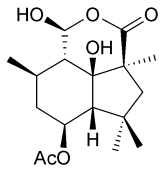
10-Oxodihydrobotrydial **(8)**



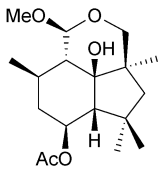
10-Dehydroxydihydrobotrydialone **(9)**



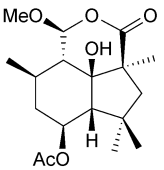
10-Epidihydrobotrydial **(10)**



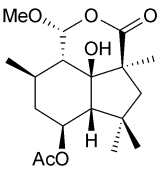
Dihydrobotrydialone **(11)**



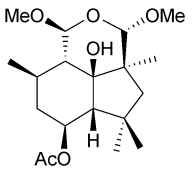
O-Methyldihydro-botrydial **(12)**



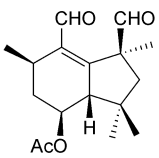
β-*O*-Methyldihydro-botrydialone **(13a)**



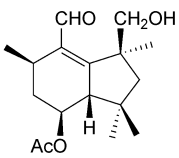
α-*O*-Methyldihydro-botrydialone **(14)**



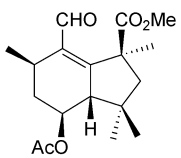
15 -Methoxy-*O*-methyldihydro-botrydial **(15)**



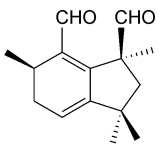
Botryendial **(16)**



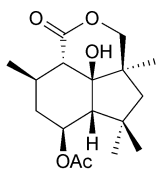
Botryenalol **(17)**



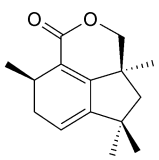
Methyl acetyl botryenaloate **(18)**



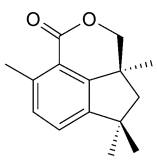
Botrydienal **(19)**



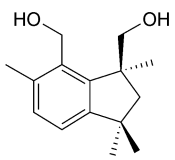
Norbotrydialone acetate **(20)**



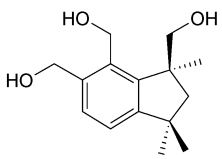
10-Oxodihydrobotry-1(9),4(5)-diendial **(21)**



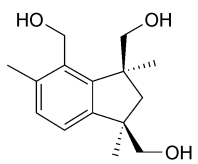
10-Oxodehydrodihydro-botrydial **(22)**



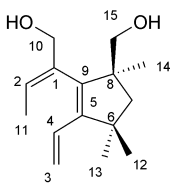
Dehydrobotrydienol **(23)**



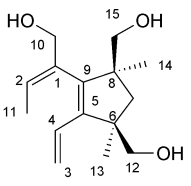
11-Hydroxydehydro-botrydienol **(24)**



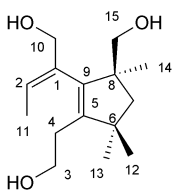
12-Hydroxydehydro-botrydienol **(25)**



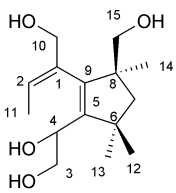
Secobotrytriendiol **(26)**



Secobotrytriene-10,12,15-triol **(27)**

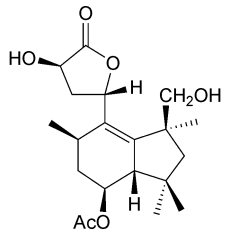


Secobotrydiene-3,10,15-triol **(28)**

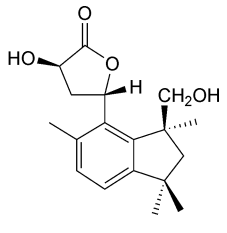


Secobotrydiene-3,4,10,15-tetraol **(29)**

Both *B. cinerea* and *B. squamosa* produce compounds (4β-acetoxytetrahydrobotryslactone (**30**),[18] and botryslactone (**31**)[20]) with a botryane skeleton and a five-membered lactone ring moiety at C-10. The biosynthetic origin of carbons C-16, C-17, and C-18 in compounds **30** and **31** may arise from the condensation of a unit of acetate, a methyl unit and the corresponding botryane, drawing a parallel with the biosynthesis of the co-occuring botcinolide metabolites.[10f]

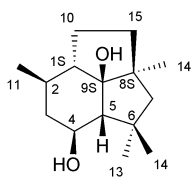


4β-Acetoxytetrahydrobotryslactone **(30)**



Botryslactone **(31)**

Biosynthetic experiments implied the formation of presilphiperfolane precursors of the botryanes by *B. cinerea.* Subsequent careful examination of the fungus revealed the presence of the probotryanes possessing this skeleton. Compounds **32**, **33** and **34** were isolated after 3 days' fermentation, the 10β- and 15α-alcohols (**33** and **34**) were present after 5 days, and after a longer period only the 4β-acetoxy-9β,10β,15α-triol (**3**) was present, indicating a biosynthetic sequence involving these compounds.[15a,21]



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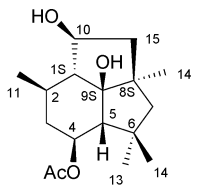

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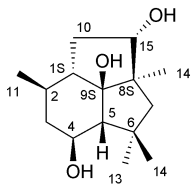

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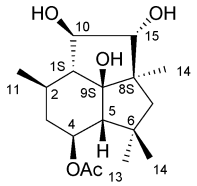


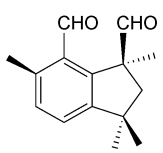
3

position numbering derived from botrydial

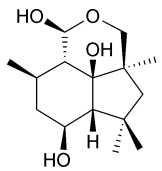
Compounds **19** and **35–37** have been found as sesquiterpenoid botryane metabolites of *B. squamosa.*[22–24]

Sesquiterpenoids with a botryane skeleton (compounds **39–48**) and a probotryane compound (**49**) have also been isolated from a marine fungus, a *Geniculosporium* species which was associated with a red alga, a *Polysiphonia* species obtained from the Baltic Sea.[25]

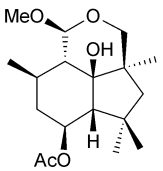
The first report on compounds with the presilphiperfolane skeleton (probotryanes) is due to Bohlmann and associates, who isolated a sesquiterpene alcohol, presilphiperfolan-8β-ol (**2**), from plant sources (*Eriophyllum staechadifolium* and *Flourensia heterolepis*).[26] Subsequently, this natural product,[27] as well as its oxidative metabolites (compounds **51–54**) have been found in other plant sources (5β,8β-diacetoxy-2β-angeloyloxypresilphiperfolane (**51**) from *Senecio anteuphorbium*,[28] *Senecio imparipinnatus*[29] and *Senecio runcinifolius*;[30] 2β-angeloyloxy-8β-hydroxypresilphiperfol-5-one (**52**) from *Ursina nana*[28,31] and *Senecio coronatus*;[32] 2β-angeloyloxypresilphiperfolane-5β-8β-diol (**53**) from *Senecio hadiensis*;[33] and 2β-tigloyloxypresilphiperfolane-5β-8β-diol (**54**) from *Senecio hadiensis*[33]).



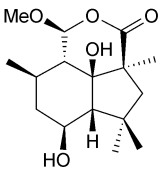
Dehydrobotrydienal **(35)**



Deacetyldihydrobotrydial **(36)**



O-Methyldihydrobotrydial **(37)**

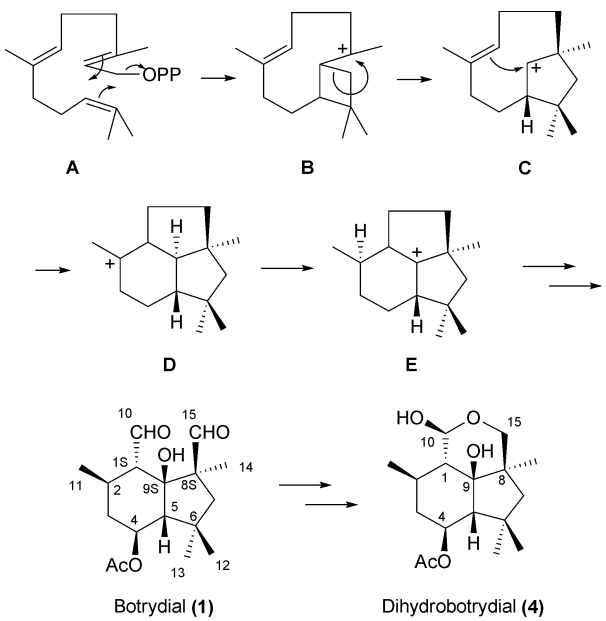


Deacetyl-*O*-methyldihydro-botrydialone **(38)**

8α-Presilphiperfolan-9-β-ol (**55**), which differs in stereochemistry at C-8 and the position of the tertiary hydroxyl group, occurs in the essential oil of *Artemisia lacinata* Willd[34] and *Artemisia chamaemelifolia*,[35] and its structure was confirmed by total synthesis of (±)-**55**.[34] This compound shows antifeedant activitiy against the chrysomelid *Leptinotarsa decemlineata* and the aphid *Diuraphis noxia.*[36]

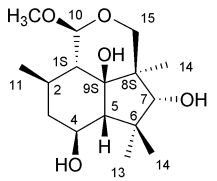
4 Biosynthesis and metabolism

Although the botryanes have an apparently non-isoprenoid structure, their sesquiterpenoid origin was established by the incorporation of [^{14}C]-farnesyl pyrophosphate into dihydrobotrydial (**4**) to the extent of 0.33%. The botryane carbon skeleton could be formed by one of a number of different foldings of farnesyl pyrophosphate (**A** in Scheme 1). These were distinguished by the

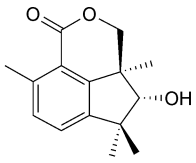


Botrydial **(1)** Dihydrobotrydial **(4)**

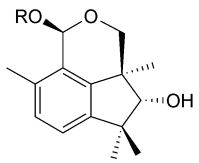
Scheme 1



7-Hydroxy-10-methoxy-deacetyldihydrobotrydial **(39)**



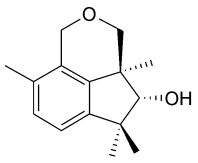
7-Hydroxy-10-oxodehydro-dihydrobotrydial **(40)**



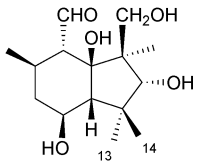
7,10-Dihydroxydehydro-dihydrobotrydial **(41)** R = H

7-Hydroxy-10-methoxydehydro-dihydrobotrydial **(42)** R = CH_3

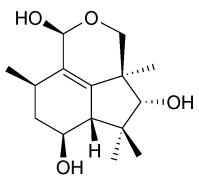
7-Hydroxy-10-ethoxydehydro-dihydrobotrydial **(43)** R = CH_2CH_3



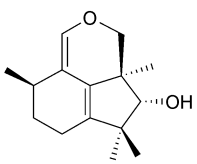
7-Hydroxy-10-dehydroxydehydro-dihydrobotrydial **(44)**



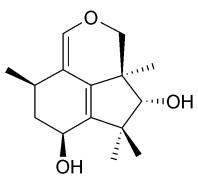
7-Hydroxydeacetyl-botryenalol **(45)**



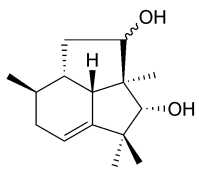
7,10-Dihydroxydeacetyl-dihydrobotrydial-1(10)-ene (**46**)



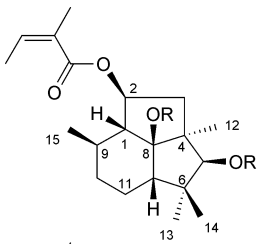
4,10-Didehydroxy-7-hydroxydeacetyl-dihydrobotrydial-1(10),5(9)-diene (**47**)



7-Hydroxy-10-dehydroxydeacetyl-dihydrobotrydial-1(10),5(9)-diene (**48**)

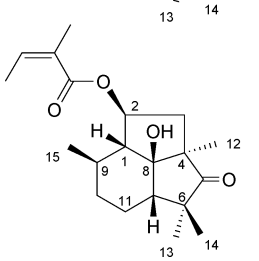


15-Hydroxy-14-aldehyde probotryan-4(5)-ene (**49**)

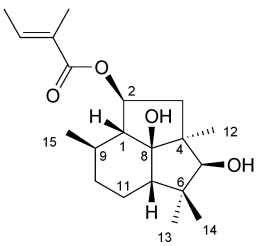


5β,8β-Diacetoxy-2β-angeloyloxy-presilphiperfolane **(51)** R = Ac

2β-Angeloyloxy-presilphiperfolane-5β,8β-diol **(53)** R = H

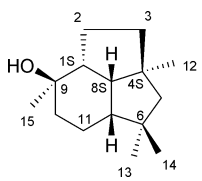


2β-Angeloyloxy-8β-hydroxy-presilphiperfol-5-one **(52)**



2β-Tigloyloxy-presilphiperfolane-5β-8β-diol **(54)**

position numbering as given by Bohlmann



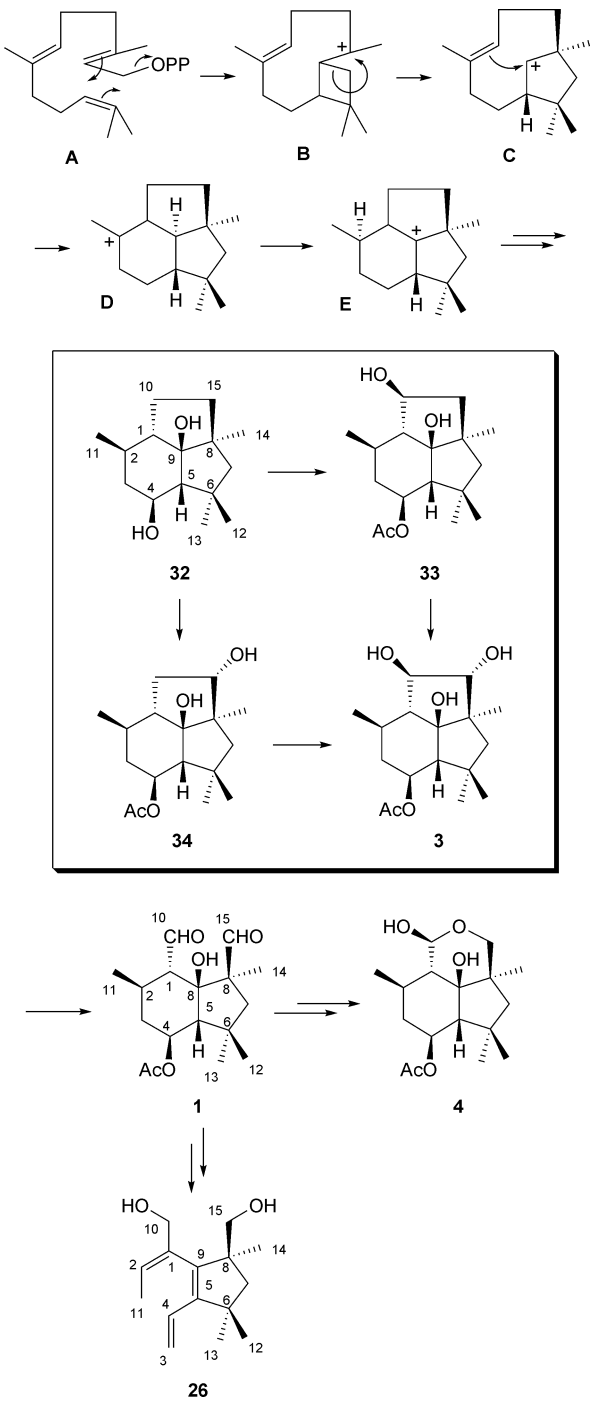
Presilphiperfolan-9β-ol (**55**)

position numbering as given by Bohlmann

labelling pattern arising from feeding of [1,2-$^{13}C_2$]-acetate, [4,5-$^{13}C_2$]-mevalonate and the induced couplings arising from a pulse-feeding experiment of [1-^{13}C]-acetate to *B. cinerea*.[37] The labelling patterns of the resultant dihydrobotrydial (**4**) were consistent with the folding of farnesyl pyrophosphate shown in Scheme 1. This is reminiscent of the biosynthetic scheme leading to the formation of caryophyllene.

The stereochemical fate of the mevalonoid hydrogen atoms shed further light on this process. All three [4(*R*)-4-^{3}H]-mevalonoid hydrogen labels were incorporated into dihydrobotrydial (**4**). However, one the centres (C-9) that would be labelled by C-4 of mevalonate carries a hydroxyl group rather than a hydrogen atom. The proposed biosynthetic scheme envisaged a rearrangement leading to the appearance of a 4(*R*)-4-MVA hydrogen atom at C-2 and the generation of a secondary methyl group. The rearrangement could take the form of two 1,2-shifts (H-9 to C-1; H-1 to C-2) or a direct 1,3-shift (H-9 to C-2). [4-^{2}H,4-^{13}C]-Mevalonic acid was fed to *B. cinerea* at a concentration such that only one labelled mevalonate unit was incorporated into each botrydial (**1**) that was biosynthesized. The retention of two ^{2}H–^{13}C couplings established that a 1,3-hydrogen shift had occurred. Studies with $H_2{}^{18}O$ revealed that the hydroxyl group at C-9 originated from water, consistent with the discharge of a presilphiperpholane C-9 cation (**E** in Scheme 1).[38]

This biosynthetic scheme envisages the cleavage of the probotryane–presilphiperfolane skeleton. The stereochemistry of this process was studied using stereospecifically labelled mevalonates. The stereochemistry of labelling of farnesyl pyrophosphate (**A**) from [2(*R*)-2-^{3}H]- and [5(*R*)-5-^{3}H]-mevalonate is known from studies on sterol biosynthesis. Evidence for the formation of botrydial (**1**) built on this knowledge. Whereas botrydial (**1**) is efficiently converted to dihydrobotrydial (**4**) (32% incorporation), the reverse transformation is significantly less efficient (1.09%). Furthermore, one hydrogen atom at C-15 of dihydrobotrydial (**4**) is of a non-mevalonoid origin. These results suggest that botrydial (**1**) is the precursor of dihydrobotrydial (**4**). The stereochemistry of the cleavage of the probotryane C-10–C-15 bond to form the dialdehyde followed from the incorporation of the stereospecifically labelled mevalonates. Biosynthetic labelling experiments showed that the C-10 hydrogen atom of dihydrobotrydial (**4**) was derived from the [5-(*R*)-^{3}H] mevalonoid hydrogen, and that C-15, which bore only one [2-$^3H_2$]-mevalonoid hydrogen, retained the [2-(*R*)-2-^{3}H] label.[38] Thus the dialdehyde was probably formed by the cleavage of a *trans*-probotryane 10β:15α glycol. Subsequent thorough investigation of *B. cinerea* revealed the presence of the probotryane–presilphiperfolane metabolites **32–34** and **3**.[21] The structures of these compounds showed that the probotryane skeleton is hydroxylated in two steps to give the glycol **3** with the above proposed stereochemistry (Scheme 2).

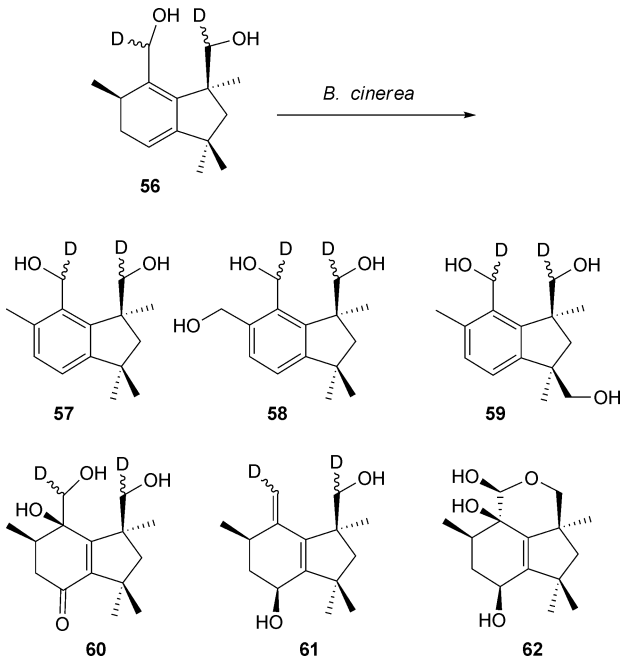


Scheme 2

Although the cleavage of the C-10–C-15 bond may take place by a direct enzymatic process, there is the possibility of neighbouring group participation from the C-9 hydroxyl group. Dehydrogention of the *cis*-9β,10β-diol may afford a transient 9:10 epidioxide, which could then undergo an acid-actalysed O–O fragmentation, leading in turn to fission of the C-10–C-15 bond and the formation of botrydial (**1**).

Botrydial (**1**) is metabolized to a range of compounds. Fungal growth ceases when the concentration of botrydial (**1**) reaches a particular level, and addition of botrydial (**1**) to fermentation broths also leads to a cessation of growth. The fungus then transforms botrydial (**1**) to the less active phytotoxins, including dihydrobotrydial (**4**) and secobotrytrienediol (**26**). Fungal growth resumes after this detoxification process has taken place.[39]

Labelling studies with [1-^{13}C]- and [1,2-$^{13}C_2$]-acetate established that the secobotrytrienediol (**26**) was biosynthesized from farnesyl pyrophosphate by the botryane pathway.[19] Whereas botrydial (**1**) was converted to the secobotrytrienediol (**26**), deuteriated botrydiendiol (**56**) was not converted into the *seco* compound but was converted into a range of aromatic metabolites (**57–59**) and to compounds that were hydroxylated at C-1 (**60**, **62**) (Scheme 3).[40]

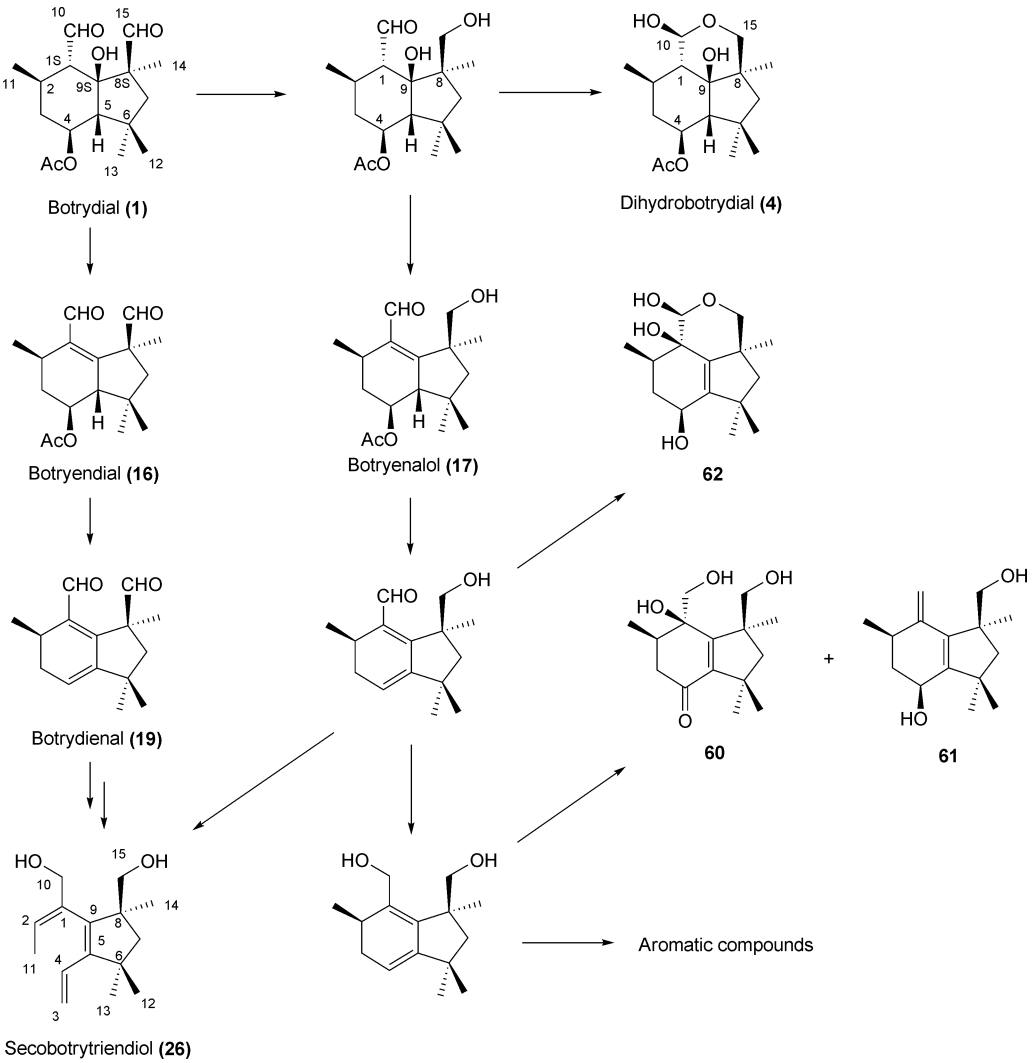


Scheme 3

The pattern of metabolites isolated was dependent on the pH of the medium. The cleavage of the diene to form a triene probably takes place with C-10 at an aldehyde oxidation level.[39] The formation of the aromatic metabolites may involve a dehydrogenation, whilst the introduction of the hydroxyl group at C-1 (compounds **60–62**) may involve the addition of oxygen to the dienes and the cleavage of an epidioxide. These metabolic steps are reminiscent of the chemistry of the diene of ergosterol.[41a] The facility with which botrydial (**1**) undergoes biodegradation to a range of compounds suggests that it may not persist in the environment or in the food chain (Scheme 4).[40]

Recently, the functional characterization of gene *bcbot1*, encoding a P450 monooxygenase, was reported, and provides evidence that it is involved in the botrydial pathway. This is the first botrydial biosynthetic gene identified.[41b]

Deletion of *bcbot1* indeed confirmed the participation of its gene product in botrydial biosynthesis. Mutants of three different *B. cinerea* strains lacking a functional copy of the gene did not show any botrydial (**1**) production, and the pathway intermediate 4β-acetoxyprobotryane-9β,10β-diol (**33**) accumulated in the knock-out mutants, which strongly suggests that the gene was involved in hydroxylation of the C-15 carbon atom of the botryane skeleton. Deletion of the gene in the strain caused reduction in virulence on bean plants, detached tomato leaves and tomato fruits, although



Scheme 4

no major influence on growth on agar plates could be detected. A synergistic effect on virulence between both classes of toxins, botryanes and botcinolides was observed.[41b]

Some chemical studies of the cyclization of caryophyllene (**63**) have been modelled on this biosynthesis. The complex acid-catalysed cyclization reactions of caryophyllene (**63**) have been reviewed.[42] Most products such as the clovanes arise by attack on the strained endocyclic double bond. However, the cyclobutylcarbinyl ring expansion of a caryophyllene-8-yl cation (**B**), implicit in botryane biosynthesis (Scheme 1), has been encountered on a number of occasions. Solvolysis of the norcaryophyllene 8-*p*-toluenesulfonate (**66a**), which was prepared from caryophyllene oxide (**64**), gave a nor-8α-presilphiperfolane-9β-ol (nor-**51**) (Scheme 5).[43] Rearrangement of isocaryophyllene (**65**) with ferric chloride gave two sets of products, including the tricyclic alkene (**68**) and the bicyclic compound (**67**) (Scheme 6).[44]

5 Biological activity

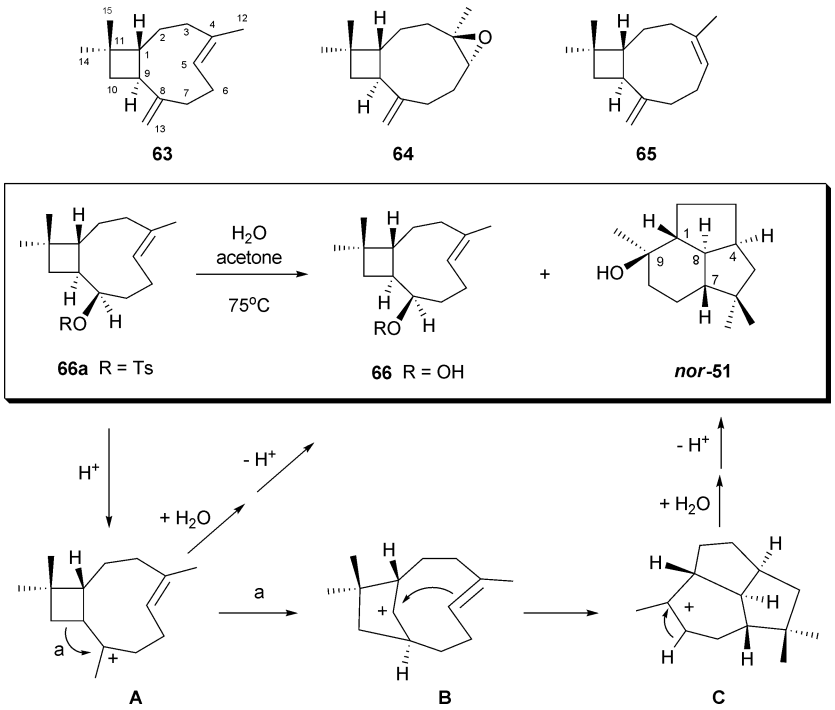
5.1 Phytotoxicity

Botrydial (**1**) is the primary phytotoxic metabolite of *B.cinerea.* It produces lesions on tobacco and bean leaves when applied to leaf discs[45] and to intact plants[9] at a concentration of 1 ppm. Botrydial has been detected in plants infected with *B. cinerea.*[9a] Dihydrobotrydial (**4**) is less active.

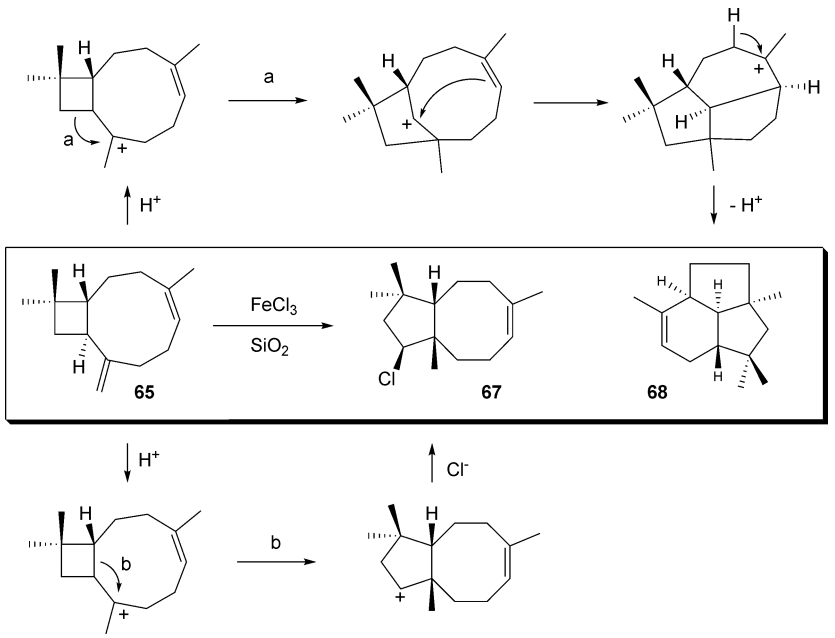
The phytotoxicity of a series of derivatives is given in Table 1. A number of generalizations can be made. The oxidation level at C-10 and C-15 is very important. Compounds that contain an aldehyde or masked aldehyde at both C-10 and C-15 show the highest activity, whereas the esters and the alcohols are either inactive or only poorly active. The relative stereochemistry of the aldehydes is also important. Both botrydial (**1**) and the 8,9-epimer (**1a**) in which the aldehyde groups can become co-planar are active, whereas the C-l epimers which do not have this co-planarity are much less active (Fig. 1). It is possible that this activity is associated with the formation of a bis-condensation product with an amine. The probotryanes did not show any activity.

5.2 Antibiotic and cytotoxic activity

Botrydial (**1**) showed significant antibiotic activity against *Bacillus subtilis*[15b] and *Pythium debaryanum*,[1] as well as high cytotoxic activity against various cell lines.[46] 8,9-Epibotrydial (**1a**) and botrydiendial (**19**) were also active against *B. subtilis.*[15b] Antibacterial



Scheme 5



Scheme 6

activity against *Escherichia coli* and *Staphylococcus aureus* was also shown by botrydiendial (**19**) and dehydrobotrydiendial (**35**).[22] In general, antibiotic activity was also associated with the presence of the co-planar dialdehyde group. Compounds with other functional groups were much less active.

The cytotoxic activity of a large number of naturally occurring botryanes and synthetic derivatives has been evaluated against a series of cell lines.[46] The results of this study showed that the presence of the 1,5-dialdehyde was important for activity and that this required the *S* configuration at C-1.

6 The development of novel antifungal agents

Plant pathogens such as *B. cinerea* cause serious economic losses.[47] There is a considerable need to develop fungicides with a novel mode of action to combat resistant strains of organisms[48] and with specific rather than general antifungal activity.[49] Strains of *B. cinerea* that are resistant to common fungicides have begun to appear.[50] A further aim is produce novel fungicides that do not impede the role of beneficial organisms[51] in plant development and which do not persist in the food chain. Part

Table 1 Phytotoxic activity for compounds isolated from *Botrytis cinerea*

Compound	Phytotoxicity (*in vitro*)	Ref.	Phytotoxicity (*in planta*)	Ref.
Botrydial (**1**)	Strong	9*b*,15*b*,45	Strong	9*b*
8,9-Epibotrydial (**1a**)	Strong	9*b*,15*b*,16	Strong	9*b*
1-Epibotrydial (**1b**)	Moderate	9*b*,15*b*	Moderate	9*b*
1,8,9-Epibotrydial (**1c**)	Low	15*b*	—	—
Dihydrobotrydial (**4**)	Low	9*b*,16	Low	9*b*
Botrydienal (**19**)	Strong	9*b*,16	Strong	9*b*
Botryendial (**16**)	Strong	9*b*,16	Strong	9*b*
Methyl botryolate acetate (R = Me) (**6a**)	Inactive	16	—	—
10-Epidihydrobotrydial (**10**)	Inactive	16	—	—
Methyl acetylbotryenaloate (**18**)	Inactive	16	—	—
10-Dehydroxydihydrobotrydialone (**9**)	Inactive	16	—	—
Secobotrytriendiol (**26**)	Inactive	19	—	—
Secobotrydiene-3,10,15-triol (**28**)	Inactive	19	—	—
Secobotrydiene-3,4,10,15-tetraol (**29**)	Inactive	19	—	—
Secobotrytriene-10,12,15-triol (**27**)	Inactive	19	—	—

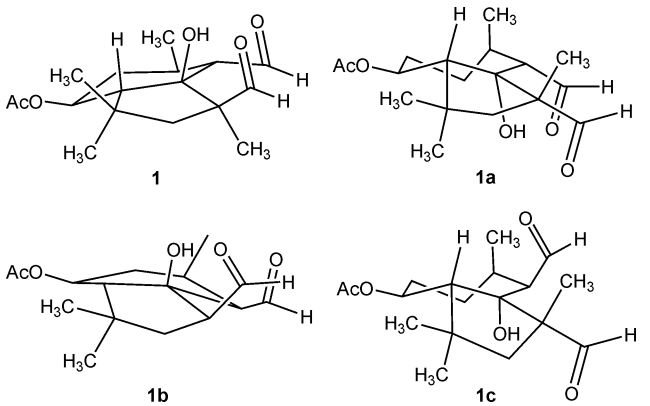


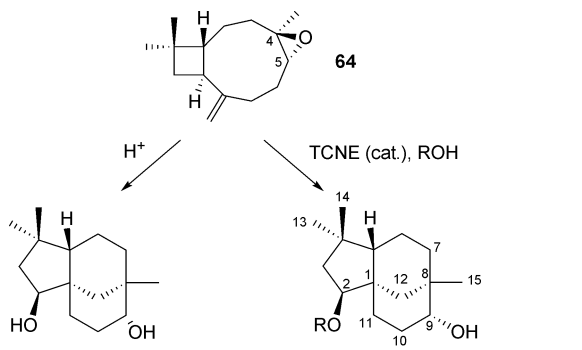
Fig. 1 Comparison of the spatial arrangement of carbonyl and hydroxyl groups in compounds **1–1c**.

of the interaction of *B. cinerea* with a host plant involves the action of the low molecular weight phytotoxin, botrydial, on the plant.[9] For example, the response of a plant to a fungal infection may involve the production of a phytoalexin as an antifungal agent.[52] Different plants produce different phytoalexins. A fungus which is a successful plant pathogen on a particular species often has the ability to metabolize and detoxify the species-specific phytoalexin. Thus, pathogenic strains of *B. cinerea* can metabolise the phytoalexin resveratrol produced by grapes.[53] Furthermore, recent evidence suggests that this process is more complex, and that resveratrol is a pro-antifungal agent, as some of the metabolites are more toxic to the fungus than resveratrol itself.[54] Resistance in plants has been achieved by transferring the genetic information for making a different phytoalexin between plants. Stilbene synthase was transferred from grapes into tobacco, conferring some resistance against pathogens to the tobacco.[55] The use of alien or modified phytoalexins thus provides a potential species-specific method of controlling plant pathogens, especially when gene therapy is not exempt from complications.[56] Phytoalexins are also produced as a consequence of the use of chemical elicitors like benzothiadiazole,[57] employed to unleash in plants the complex phenomenon of systemic-acquired resistance (SAR).[58] In this context it is worth noting that a number of phytoalexins such as rishitin are sesquiterpenoids.

Secondly, there is evidence that the production of characteristic secondary metabolites during the idiophase of fungal growth has a limiting effect on fungal growth. Botrydial (**1**) has this effect on the growth of *B. cinerea*.[39] Furthermore, the addition of the metabolite during the earlier growth phase of the organism may have the consequence of restricting its development. A method of fungal control which has been explored in the work which will be discussed below is to use a non-phytotoxic analogue of botrydial (**1**) or a biosynthetic intermediate to produce the same effect.

A third approach is to block the biosynthesis of botrydial (**1**) to prevent the fungus from exerting its pathogenicity. The effect of compounds related to the biosynthetic intermediates such as the presilphiperfolanes has been examined in this context. The sesquiterpenoid caryophyllene (**63**) is readily available from the clove tree, *Eugenia caryophyllata*. Caryophyllene and a number of its relatives are already present in the food chain and do not display significant human toxicity. Several of the cyclization products of caryophyllene are reminiscent of botrydial biosynthetic intermediates. Other readily available eremophilanes such as valencene, obtained from citrus fruit, have a similarity to phytoalexins.

The effect on the growth of the fungus of a number of compounds related to botryane biosynthetic intermediates has been studied. Treatment of caryophyllene oxide (**64**) with aqueous acid leads to the formation of clovane-2β,9α-diol (**69**).[59] If the Brønsted acid is replaced by the π-acid catalyst tetracyanoethylene and an alcohol is used as a solvent, solvolysis of the caryophyllene (**64**) oxide leads to the formation of 2β-alkoxyclovan-9α-ols **70–75** (Scheme 7).[60]



Scheme 7

A series of clovane derivatives were prepared (see Scheme 7) and incubated with *B. cinerea*.[61] The effectiveness of the inhibition of the growth of the fungus was evaluated and found to be of the order of 80%, for compound 2β-methoxyclovan-9α-ol (**70**), at a concentration of 100 ppm, after 3 days. Compound **70** was metabolized over a period of 5 days by the fungus to 2β-methoxyclovan-9-one (**76**), 2β-methoxyclovan-9β-ol (**77**), clovane-2β,9α-diol (**69**), clovane-2β,9β-diol (**78**) and 2β-methoxyclovane-9α,14-diol (**79**).[62] These metabolites were only poorly active (Fig. 2), suggesting that the hydroxylations are detoxification processes. The metabolic inversion of configuration at C-9 only proceeded in the direction of the 9α-alcohol (**70**) to the 9β-alcohol (**77**), although the 9-ketone **76** is a metabolite of the 9β-alcohol **77** (Table 2). The reduction at C-9 follows the Prelog rule for the microbial asymmetric reduction of ketones.[63] The facile dealkylation of the 2β-methyl ether **70** to form the 2β,9α- and 2β,9β-diols (**69** and **78**, respectively) would afford an easy route to the 2,9-dione clovane-2,9-dione (**80**). As a 1,5-diketone, compound **80** undergoes further degradation and does not persist in the medium. The existence of a microbial detoxification pathway for these fungistatic agents suggests that they might not persist in the environment for a prolonged period. An increase in the chain length of the ether at C-2, and the presence of certain functional groups within it, increased the effectiveness of the inhibitors.[64,65]

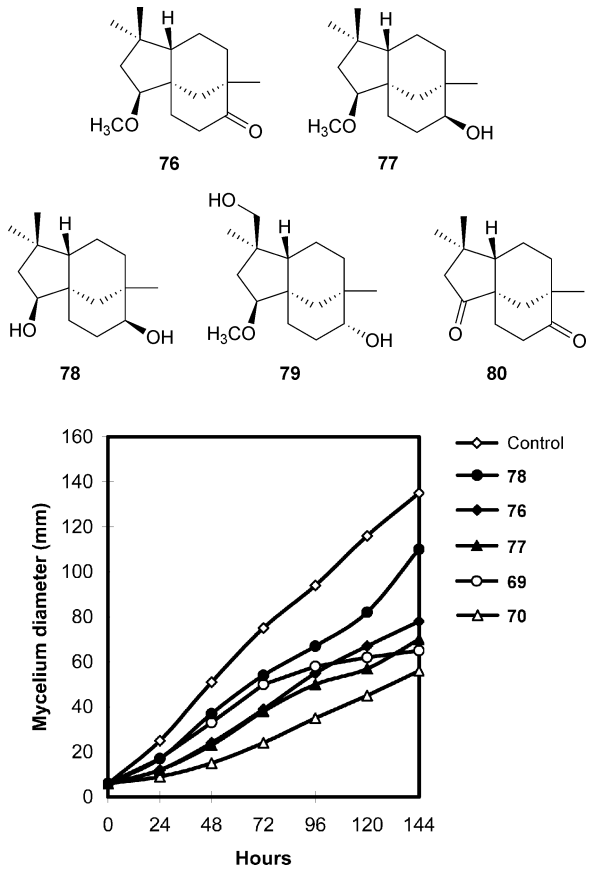
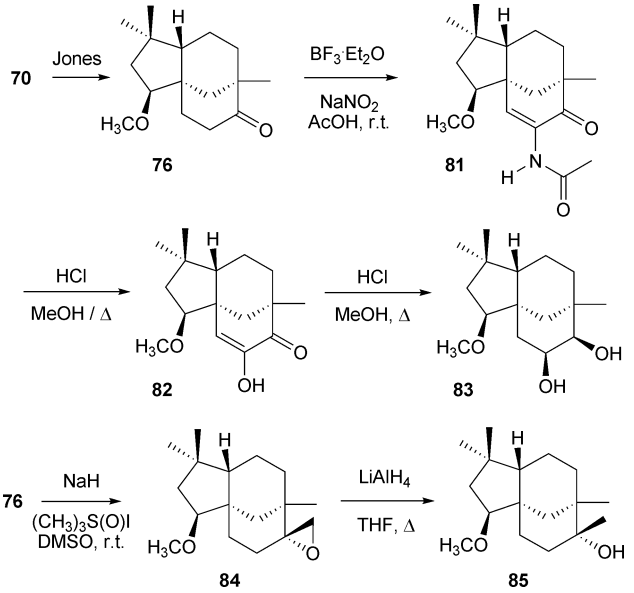


Fig. 2 Comparison of fungal growth inhibition (*B. cinerea*) between compounds **69**, **70**, **76**, **77** and **78** at a 100 ppm dose level.

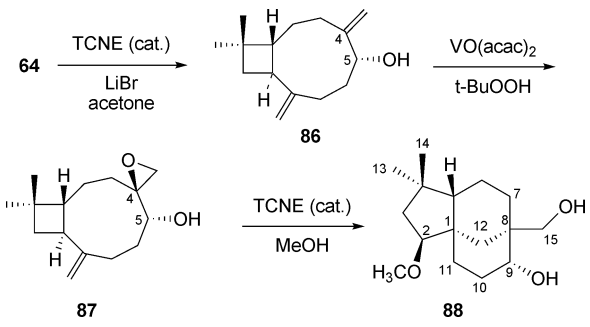
Some more highly oxidized clovanes were also prepared (see Schemes 8–10). However, there was a general decrease in activity when further hydroxyl groups were introduced onto the clovane

Table 2 Metabolites of clovanes **70**, **76**, **77** and **80** by *B. cinerea*

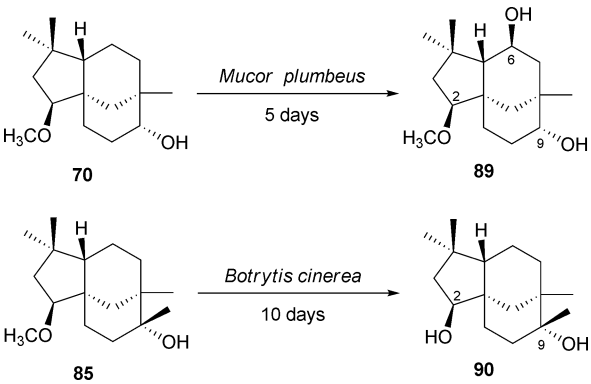
Substrate	Metabolites
70 (5 days)	**69**, **76**, **77**, **78**, **79**
70 (9 days)	**69**, **76**, **77**, **78**
70 (16 days)	**69**, **78**
76 (10 days)	**77**, **78**, **79**
77 (10 days)	**76**, **78**, **79**
80 (4 days)	No clovane metabolites



Scheme 8



Scheme 9



Scheme 10

skeleton, particularly if the additional groups were adjacent to C-9 (Fig. 3 and 4).

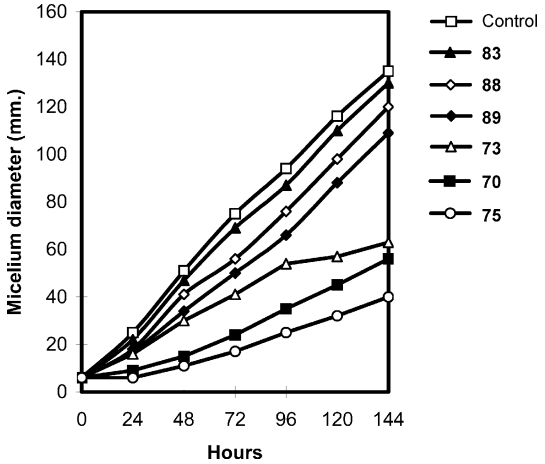


Fig. 3 Comparison of fungal growth inhibition (*B. cinerea*) between compounds **70**, **73**, **75**, **83**, **88** and **89** at a 100 ppm dose level.

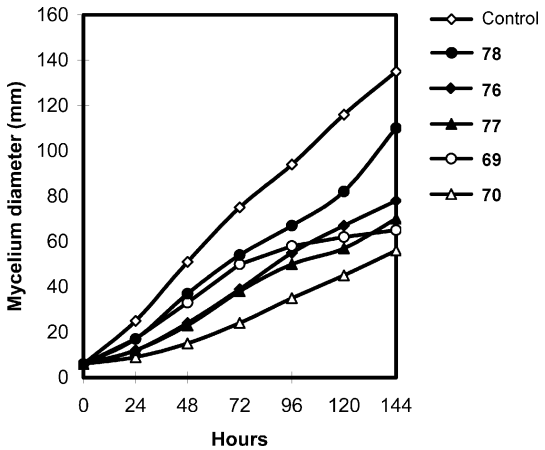


Fig. 4 Comparison of fungal growth inhibition (*B. cinerea*) between compounds **69**, **70**, **85** and **90** at a 100 ppm dose level.

Further sesquiterpene derivatives which have been studied in the context of inhibition of the growth of *B. cinerea* include compounds with the caryophyllane,[66] ginsane,[67] patchoulane,[67b,68] isoprobotryane,[44,67b] 1-epiprobotryane,[69] norprobotryane (norpresilphiperfolane),[70] cedrane,[67b] globulane,[67b] daucane[71] and eremophilane[72] skeletons. The hydroxylation and detoxification of these compounds by *B. cinerea* has been studied.[66–68,70–72] The activities of some selected compounds (**91**–**96**) are presented in Table 3.

In some cases, the detoxification involved hydroxylation on one of the *gem*-dimethyl groups (see compounds **93a–c**, derived from isoprobotryan-9α-ol (**93**),[67b] and **94a**, derived from globulol (**94**)[67b]). In other cases, the oxidation pattern was on the opposite side of the molecule to the *gem*-dimethyl group, specially if a tertiary alcohol was present in a central position in the molecule (see compounds **91a,b**, derived from ginsenol (**91**),[67a] and **92a–c**, derived from patchoulol (**92**)[68]). This latter hydroxylation pattern is reminiscent of that one shown in 4β-acetoxyprobotryane-9β,10β,15α-triol (**3**) at C-10 and C-15. Some readily available sesquiterpenoid derivatives of this type have been examined in field trials in Spain for the successful control of *Botrytis cinerea* in commercial crops.

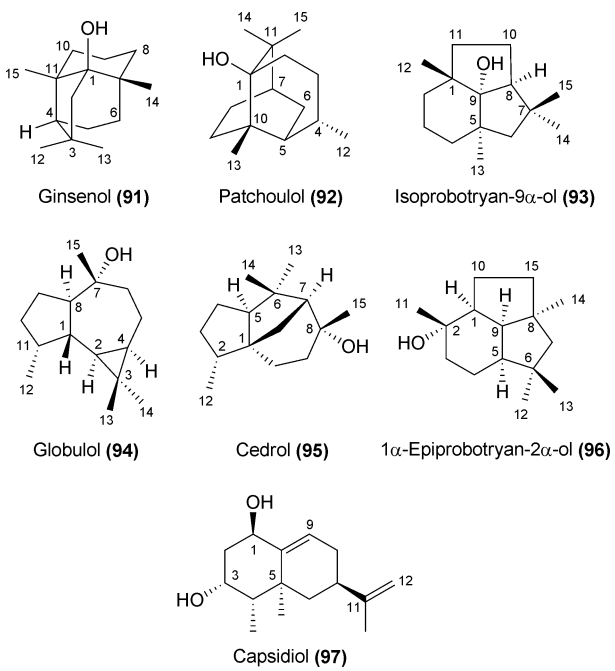
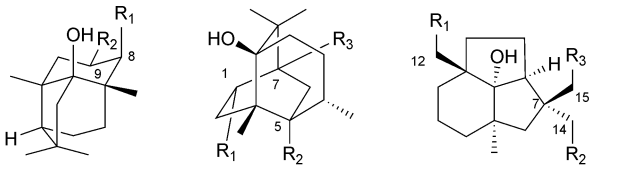


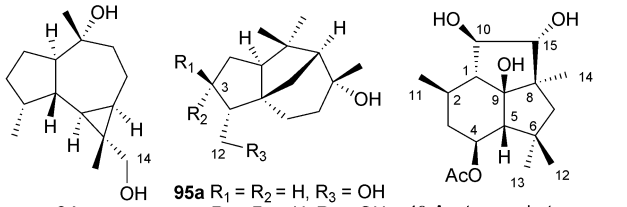
Table 3 Comparison of fungal activity *versus* molecular geometry for selected compounds

Compound	*I* (%)[a]	Atoms considered	Distance/Å
Ginsenol (**91**)	86	C-1–C-3	2.53
Patchoulol (**92**)	99	C-1–C-11	1.60
Isoprobotryan-9α-ol (**93**)	95	C-9–C-7	2.48
Globulol (**94**)	90	C-7–C-3	4.11
Cedrol (**95**)	23	C-8–C-6	2.70
1-Epiprobotryan-2α-ol (**96**)	14	C-2–C-6	4.41
4β-Acetoxyprobotryane-9β,10β,15α-triol (**3**)	—	C-9–C-6	2.41
Capsidiol (**97**)	—[b]	C-1–C-11	5.85
		C-3–C-11	6.13

[a] Percentage of inhibition of fungal growth (*B. cinerea*) at a 100 ppm dose. [b] Not available; the MIC (minimum inhibitory concentration) has been recently reported to be 10 ng per spot (see ref. [75]).



91a R_1 = H, R_2 = OH **92a** R_1 = R_2 = H, R_3 = OH **93a** R_1 = R_2 = H, R_3 = OH
91b R_1 = OH, R_2 = H **92b** R_1 = R_3 = H, R_2 = OH **93b** R_1 = R_3 = H, R_2 = OH
92c R_2 = R_3 = H, R_1 = OH **93c** R_2 = R_3 = H, R_1 = OH



94a **95a** R_1 = R_2 = H, R_3 = OH
95b R_1 = R_3 = H, R_2 = OH
95c R_2 = R_3 = H, R_1 = OH
4β-Acetoxyprobotryane-9β,10β,15α-triol **(3)**

The location and stereochemistry of a hydroxyl group is also important for biological activity. Molecular modelling studies[73] have suggested that the most active compounds may fall into two groups. There are those substances in which the distance between the carbon atom bearing the hydrophobic methyl groups and the hydroxyl group mimics the distance between C-6 and the C-9 oxygen of botrydial (**1**), dihydrobotrydial (**4**) and their precursor 4β-acetoxyprobotryane-9β,10β,15α-triol (**3**).† On the other hand, there are those in which the above-mentioned distance is similar to the distance between the *gem*-dimethyl group and the C-l hydroxyl group of the sesquiterpenoid phytoalexin, capsidiol. It is possible that one group of inhibitors may act as botryane biosynthetic mimics (especially those which possess a similar oxidation pattern to that shown in compound **3**), whilst the others are analogues of sesquiterpenoid phytoalexins. Interestingly, this distance is found in the readily available synthetic compound diisophorone, and this compound has been shown to be an inhibitor of the growth of *B. cinerea.*.[74] It is possible that the compounds which are botryane mimics may exert their biological activity either by blocking botryane biosynthesis or by mimicking the regulatory effect of idiophase metabolites whilst the fungus is still in the exponential growth phase. Either mechanism of action would provide a novel species-specific method for the control of this (and possibly other) phytopathogenic fungi.

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† Another requirement is that the hydrophobic tertiary alcohol should occupy a "central" position in the molecule; for instance, cedrol fulfils the distance requirement, but the hydroxyl group is "peripheral", and therefore has a low inhibitory activity.

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