ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 13 (2001) 77–93

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Biotransformations by *Botrytis* species

Josefina Aleu, Isidro González Collado*

Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, República Saharaui s/n, Apdo. 40, 11510 Puerto Real, Cádiz, Spain

Received 23 June 2000; accepted 18 September 2000

Abstract

Biotransformations by *Botrytis* sp. are reviewed. Various substrates and the *Botrytis* species used for the transformations are included in this review of the literature for the period 1961–1999. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Biotransformation; *Botrytis*; Phytopathogen; Fungus; Terpene

1. Introduction

Nearly 80% of all plant disease is caused by fungi and despite the extensive use of fungicides and other control measures, the financial losses due to fungal activity remain enormous.

The *Botrytis* species are polyphage parasites which are able to live either as saprophytes, feeding off decomposing organic material, or as parasites or semi-parasites and can even alternate between periods of each type of behaviour. This type of parasites produce toxins as well as wall-degrading enzymes, have a wide host range and can survive as saprophytes on dead plant tissue, although, their actual growth must occur on immature, damaged or senescent plants [1].

These species belong to the most geographically wide-spread group of plant pathogens. They are found wherever a suitable host crop is cultivated, from cold regions such as Alaska and Canada to subtropical regions such as Egypt.

Many species belonging to this genus cause serious losses to a number of commercial crops as they are implicated in numerous diseases of flowers, fruits and vegetables [1].

- *B. cinerea*: a pathogen of grapes, lettuces, tomatoes, carrots, tobacco and strawberries, among a total of more than 235 identified plant species. It is the agent responsible for the disease known as “grey mould”, so named because it produces a grey powdery mould on the crops it infects.
- *B. squamosa*: a pathogen of onions that causes the stalk to break.
- *B. allii* and *B. byssoidea*: pathogens of onions.
- *B. fabae*: a pathogen of beans which causes epidemic spots on the leaves.
- *B. tulipae* a pathogen responsible for burns on tulip and saffron flowers.
- *B. gladioli*: a pathogen of gladioli and lilies.

Fungi also have a number of beneficial uses, especially in traditional processes or arts, such as brewing

* Corresponding author. Tel.: +34-956-016-371 or +34-956-016-368; fax: +34-956-016-288.
E-mail addresses: josefina.aleu@uca.es (J. Aleu), isidro.gonzalez@uca.es (I.G. Collado).

1381-1177/01/$ – see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S1381-1177(00)00232-0

and wine making, which have existed since antiquity. Even in these traditional processes, however, modern fungal biotechnology is having a marked impact.

The ability to utilise fungi in biotechnology has been dramatically extended in recent years, mostly due to the application of new knowledge concerning molecular fungal genetics. In addition, there seems to be an increasing willingness among fungal biotechnologists to consider novel, sometimes even seemingly bizarre, uses for fungi [2].

The use of biological methods to bring about chemical reactions forms a bridge between chemistry and biochemistry. Biotransformations can, thus, be defined as the use of biological systems to produce chemical changes on compounds that are not their natural substrates. Isolated enzyme systems or intact whole organisms may be used for biotransformations. Each approach has its advantages and disadvantages. Many isolated enzyme systems are now commercially available or are relatively easy to isolate and they can be stable and easy to use, often giving single products. However, for some reactions in which a co-factor is used, the need to regenerate the co-factor can be an added complication. Whole organisms do not have this disadvantage and, although, they do tend to give more than one product, they are often cheaper to use than isolated enzyme systems [3].

Biotransformations have a number of advantages when compared to the corresponding chemical methods. The conditions for such processes are mild and in the majority of cases do not require the protection of other functional groups. In many cases, biotransformations are also enantiospecific, allowing for the production of chiral products from racemic mixtures. Furthermore, the features governing their regiospecificity differ from those controlling chemical specificity and indeed, it is possible to obtain biotransformations at centres that are chemically unreactive. Economically, some biotransformations can be cheaper and more direct than their chemical analogues and the conversions normally proceed under conditions that are regarded as ecologically acceptable. However, many of the rules that define biotransformations are not well understood and there are many chemical reactions for which there is no equivalent bioconversion. The chemist must, thus, learn to use biotransformations alongside the conventional chemical reagents [3].

Traditionally, the species of *Botrytis* have not been specifically used to produce biotransformations, indeed it is only during the last 40 years that papers have been published on this subject.

In the sixties, some studies began to appear concerning the alcohol-oxidising activity carried out by *B. cinerea* on some substrates. In 1971, Fukuda et al. [4] made "cell-free" preparations of this fungus and discovered that they oxidised primary linear chain alcohols (except methanol), primary aromatic alcohols and primary unsaturated alcohols. The resulting products were the corresponding aldehydes and an equimolar quantity of hydrogen peroxide.

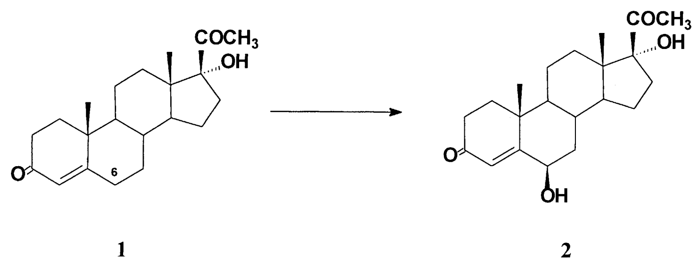
Alcohol oxidase enzymes of different specificity for the substrate have been found in a large number of micro-organisms. DeJong et al. [5] found a glucose dehydrogenase and a phenoloxidase in lyophilised extracts of *B. cinerea*, confirming that the fungus has a significantly high alcohol-oxidising activity.

In their research, Fukuda et al. [4] confirmed that primary aromatic alcohols are better substrates than aliphatic alcohols for the oxidases of *B. cinerea*. Secondary alcohols and primary alcohols with lateral chains were not oxidised, and the introduction of a polar group (halogen, amine, thiol or hydroxyl) completely deactivated the primary alcohols as substrates for the oxidases. None of the many sterols, carbohydrates or amino acids tested was oxidised by the preparation of oxidases of *B. cinerea*.

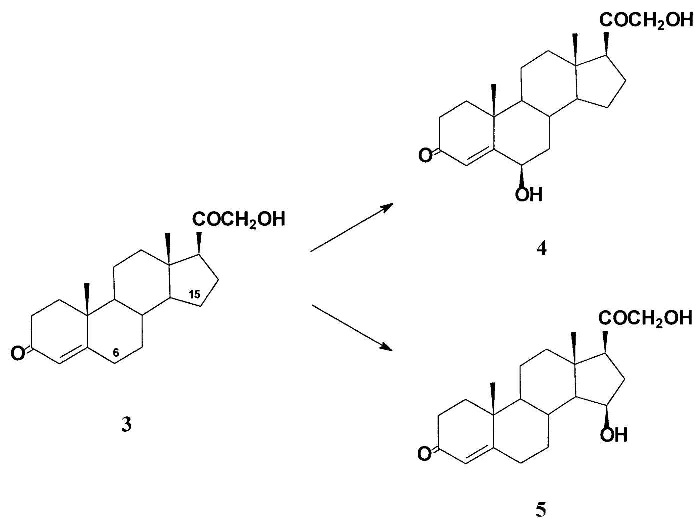
2. Biotransformations of steroids

In 1961, Shirasaka [6] studied the microbiological transformation of steroids, particularly hydroxylations, by means of *B. cinerea*. The 6β-hydroxylation of steroids by micro-organisms is a fairly common reaction, although, it normally appears to be accompanied by other reactions, particularly 11α-hydroxylation, but also 14α-, 17α- and 15α-hydroxylations.

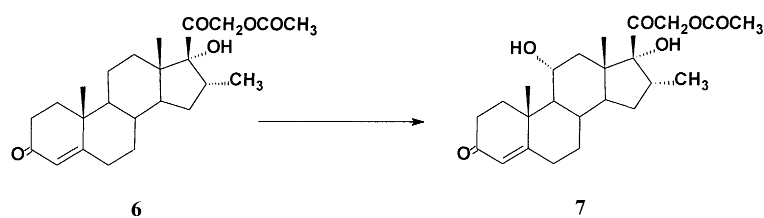
The application of *B. cinerea* to 17α-hydroxyprogesterone (**1**), deoxycorticosterone (**3**) and corticosterone as initial steroids gives rise solely to the formation of the 6β-hydroxy derivative of 17α-hydroxyprogesterone (**2**), the 15β-hydroxy derivative of corticosterone and both of the derivatives of deoxycorticosterone (**4** and **5**).



These results indicate that this fungus shows a marked specificity with respect to the substrate.



Some years later, in 1965, Holmlund et al. [7] studied the microbiological transformations of the steroid, 21-acetoxy-17α-hydroxy-16α-methylpregn-4-ene-3, 20-dione (**6**) by means of various fungi, including *B. cinerea*, which yielded the 11α-hydroxy derivative (**7**).



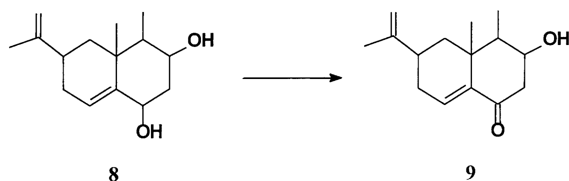
In 1980, Flesch et al. [8] found that the fungus *B. cinerea* was able to produce cholesterol after the addition of lanosterol to the culture medium and in 1983, Nespiak [9] studied the transformation of cholesterol by three species of *Botrytis*, with yields of approximately 10%. The activity varied depending on the strain used and did not appear to be related to cellular growth. The product had a keto group and contained one less hydroxyl group and was, thus, named cholest-4-en-3-one.

3. Biotransformations of sesquiterpenoids

Mono and sesquiterpenoids are primarily plant products and are well known as characteristic components

of a wide variety of essential oils. Biosynthetic pathways of terpenoids in plants were proposed by Croteau [10] and the formation of these compounds by fungi was elucidated by Collins et al. [11,12]. In addition, Tressl et al. [13] reviewed in detail the isomerisation and specific reactions of terpenoids by fungi. More recently, advanced studies were performed on the formation of terpenoids by yeast [14] and bacteria [15].

A short time later, researchers began to relate the occurrence of the biotransformations with possible detoxifications carried out by *B. cinerea*. In 1972, Ward et al. [16] studied the biotransformation of capsidiol (**8**). This compound, an antifungal sesquiterpene, is oxidised to the ketone, capsenone (**9**) by *B. cinerea* and *Fusarium oxysporum*. Capsenone (**9**) is less fungitoxic than capsidiol (**8**), therefore, its formation may be a significant mechanism of detoxification, thus, reducing the resistance of the host to the fungus.

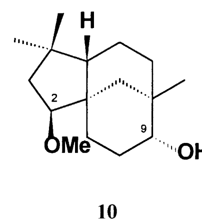


It has since been established that the formation of capsidiol (**8**) is triggered by numerous varieties of fungi in the pepper plant *Capsicum frutescens* L. Interestingly, the concentrations of capsidiol (**8**) found in the extracts of the plant are inversely related to the pathogenicity of the fungus, which may mean that this compound plays a role in the natural defences of the plant [17]. The biotransformation, therefore, would correspond to an attempt by the fungus to eliminate in some way a compound that is toxic to it by transforming it into another, relatively less toxic compound.

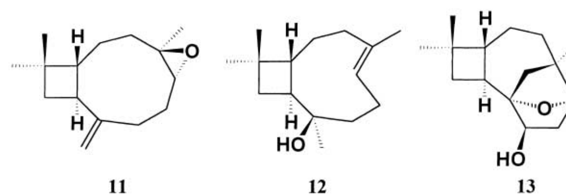
In addition, in recent years, Collado et al. [18–22] have studied the effects of *Botrytis cinerea* on the metabolism of the some sesquiterpenoids.

In 1998, these authors [18] investigated the metabolism of the fungistatic agent 2β-methoxyclovan-9α-ol (**10**) by *Botrytis cinerea*. This process yielded 2β-methoxyclovan-9-one, 2β-methoxyclovan-9β-ol, clovan-2β,9α-diol, clovan-2β,9β-diol and 2β-methoxyclovan-9β,13-diol. The effects of this fungus on the metabolism of the related compounds 2β-methoxyclovan-9-one, 2β-methoxyclovan-9β-ol and clovan-2,9-dione were also studied. Incubation of 2β-methoxyclovan-9-one with *Botrytis cinerea* yielded three products with a clovane skeleton (2β-methoxyclovan-9β-ol, clovan-2β,9β-diol and 2β-methoxyclovan-9β,13-diol). Similar tests with 2β-methoxyclovan-9β-ol also gave three clovane derivatives (2β-methoxyclovan-9-one, clovan-2β,9β-diol and 2β-methoxyclovan-9β, 13-diol). When clovan-2,9-dione was incubated with the fungus, neither unchanged material nor any metabolite was isolated from the fermentation broth.

All the major metabolites of the fungistatic compound **10** showed a much reduced biological activity when compared with the parent compound. Thus, the existence of microbial detoxification pathways for these fungistatic agents suggests that they might not persist in the environment for a prolonged period.



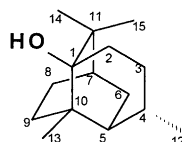
In 1999, the same authors [19,20] published the biotransformation by *Botrytis cinerea* of two compounds with the caryophyllene skeleton: caryophyllene oxide (**11**) and (8*R*)-4*E*-caryophylla-4(5)-en-8-ol (**12**). The conversion of **11** by this fungus [19] afforded 15 products. The main reaction pathways involved stereoselective epoxidation at C8/C13 and hydroxylation at C7. A rearranged compound possessing the caryolane skeleton (**13**) was also found.



Similarly, the transformation of (8*R*)-4*E*-caryophylla-4(5)-en-8-ol (**12**) [20] by *Botrytis cinerea* yielded 14 compounds. The main reaction pathways involved the isomerisation of the double bond at C4/C5 and hydroxylations at methyl groups.

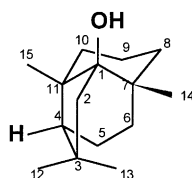
In the same year, these authors also published work on the biotransformation of some fungistatic sesquiterpenoids by *Botrytis cinerea* [21,22].

Bioconversion of patchoulol (**14**) [21] by the fungus afforded the 5-, 7- and (8*R*)-hydroxy derivatives as the major metabolites, together with a number of minor metabolites arising from hydroxylation at C2, C3, C5, C9, C13 and C14. In addition, the antifungal properties of patchoulol (**14**) against the growth of *Botrytis cinerea* were established.



14

In a subsequent paper, these authors described the biotransformation of ginsenol (**15**) [22], another fungistatic sesquiterpenoid, by *Botrytis cinerea* and also determined the antifungal properties of this compound. This transformation gave the 8- and 9-hydroxy derivatives as the major metabolites, along with the 6α- and 10β-hydroxy and 8- and 9-oxoderivatives as minor metabolites. The structure and stereochemistry of the major compound (9β-hydroxyginsenol) were established by means of X-ray crystallography.



15

In the course of the biotransformation experiments on **14** and **15**, some effects were observed on the growth of the fungus *Botrytis cinerea*. The growth of the mycelium was inhibited when the substrate was added to the broth and examination of the broth extracts showed that phytotoxic metabolites were not present in the early stages of the fermentation when the fungistatic compounds were still present. *Botrytis cinerea* was shown to have the ability to degrade products **14** and **15**, a fact which suggests that a detoxification mechanism was present.

4. Biotransformations of monoterpenoids and natural aromas

The presence of these compounds in grapes and wines has been studied by many researchers. In 1982, Shimizu et al. [23] studied the transformation of terpenoids in grape must by *B. cinerea*.

So-called “noble” wine is made with grapes botrytised with *B. cinerea* and is characterised by a very distinctive aroma. One of the steps responsible for the generation of this aroma seems to be the transformation of the terpenoids in the grapes into volatile substances by the action of *B. cinerea*, which attacks the grape skin. Nevertheless, very few articles have been published on the components of the aroma produced by this fungus.

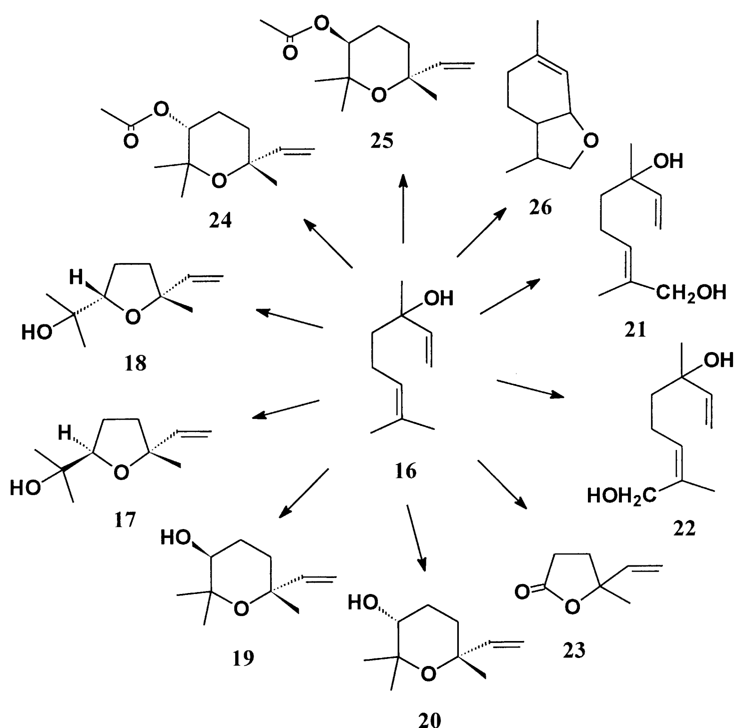
Shimizu et al. [23] investigated the volatile components of the must made from botrytised grapes and the transformation by *B. cinerea* of terpenoids in grape must supplemented with terpenes. These researchers, using a combination of gas chromatography and mass spectrometry, were able to identify a total of 28 compounds in the volatile concentrate of botrytised must.

The next stage of the study involved addition of four terpenes to separate samples of the botrytised must: linalool (**16**), furan-linalool oxide, terpinen-4-ol and α-terpineol. After incubation for 15 days, only the quantities of linalool (**16**) and terpinen-4-ol were found to have decreased. In the must to which this latter product had been added, the only terpenic compound detected after the incubation period was geranial.

In the presence of linalool (**16**), 12 terpenoids were obtained in addition to the 28 compounds found in the botrytised grape must, these were β-pinene, *trans*-furan-linalool oxide (**17**), *cis*-furan-linalool oxide (**18**), *p*-menthan-1-en-3-one, *p*-menthan-1,8-dien-9-ol, geraniol, α-terpineol, *trans*-pyran-linalool oxide (**19**), *cis*-pyran-linalool oxide (**20**) and three unidentified monoterpenes. This suggests that the fungus *B. cinerea* transforms linalool (**16**) into all of these compounds and that this transformation is one of the factors in the production of the characteristic aroma of “noble” wine.

Some years later, in 1986, this research was used by Bock et al. [24,25] as a basis for a study of the biotransformation of linalool (**16**) in grape must using

three different strains of *B. cinerea*. The use of various spectroscopic techniques led the researchers to conclude that more than 90% of the linalool (**16**) was converted into (*E*)-2,6-dimethylocta-2,7-diene-1,6-diol (**21**). Other products of the metabolisation of linalool (**16**), amounting to less than 10% of those identified, were the corresponding (*Z*)-isomer (**22**), 2-vinyl-2-methyltetrahydrofuran-5-one (**23**), the four (*E*) and (*Z*) linalool oxides in their furanoid (**17** and **18**) and pyranoid (**19** and **20**) forms, the (*E*) and (*Z*) acetates of pyranoid linalool oxides (**24** and **25**), as well as 3,9-epoxy-*p*-menth-1-ene (**26**). It was, therefore, concluded that the predominant bioconversions were ω-hydroxylations.

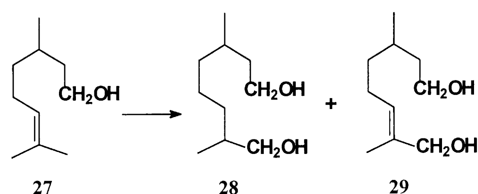


These authors observed quantitative variations in the products obtained depending on the particular strain of *B. cinerea* used. All of these volatile compounds can be considered as causing the aroma of wines botrytised with the noble rot.

A year later, the same authors [26] published another biotransformation produced by *B. cinerea*, namely, the conversion of citronellol (**27**), another terpenic alcohol, which is one of the most widely used products in commercial perfume manufacture.

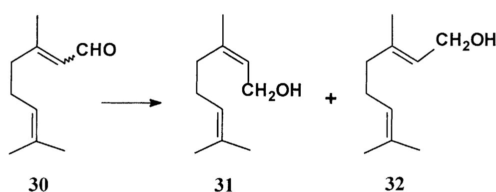
Four strains of this fungus were used with grape must as the culture medium, the main products obtained from the biotransformations were 2,6-dimethyloctan-1,8-diol (**28**) and (*E*)-2,6-dimethyloct-2-en-1,8-diol (**29**). Smaller quantities of other compounds were also obtained, including 2,6-dimethyloctan-2,8-diol, two *p*-menthan-3,8-diol isomers, (*Z*)-2,6-dimethyloct-2-en-1,8-diol, isopulegol, 2-methylhept-2-en-6-one-1-ol and 2-methyl-γ-butyrolactone. The results obtained were significantly dependent on the strain used and indeed, one of the strains did not produce a single biotransformation metabolite. In general, the percentage of metabolites formed was low, indicating that citronellol (**27**) was probably incorporated into biomass and/or degraded into CO_2 and H_2O.

It seems reasonable to assume that with must as the medium and under optimum growth conditions, **27** was transformed predominantly by means of ω-oxidations, leading to **29**, which then was transformed into **28** by reduction of the 2,3-double bond.



In 1987, as a continuation of this line of research into the bioconversion of terpenes found in wines made from botrytised grapes, the same authors [27] published the biotransformation of the monoterpene citral (**30**) by *B. cinerea*. This α,β-unsaturated terpene aldehyde is particularly important in perfumery and in citrus flavours and aromatised products [28].

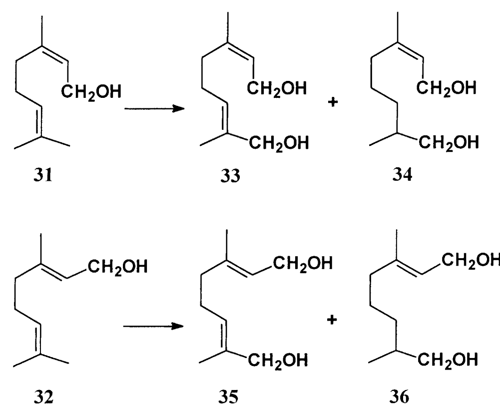
The experiments they described involved four different strains of *B. cinerea* in three different media: grape must, a synthetic medium and a mixture of the two. When must was used, a complete metabolisation of citral (**30**) was observed with no evidence of any volatile product, probably due to the incorporation of the substrate into the biomass or its degradation into CO_2 and H_2O. With the synthetic medium, the main volatile products of bioconversion found were nerol (**31**) and geraniol (**32**). In addition, the following products were identified in smaller quantities: (*E*,*Z*)-2,6-dimethylocta-2,6-dien-1, 8-diol and its (*E*,*E*)-isomer, 2-methylhept-2-en-6-one, 2-methylhept-2-en-6-ol, 2-methylhept-2-en-6-one-1-ol and 2-methyl-γ-butyrolactone. When the must/synthetic mixture was used as the medium, a lower yield of the two isomers, i.e. (*E*,*Z*) and (*E*,*E*), was obtained while the other products were found in increased quantities. Quantitatively, the results were significantly dependent on the particular strains used.



It can, thus, be concluded that the ω-hydroxylation was one of the predominant metabolic steps. However, oxidation at C1 and to a certain extent, hydrogenation of the double bonds were also observed.

In 1988, the same authors published the results of their studies on other bioconversions of monoterpenic alcohols present in botrytised grape must [29,30]. The study included four different strains of *B. cinerea* and three different culture media: grape must, a synthetic medium and a mixture of the two.

The study of the biotransformation of nerol (**31**) and geraniol (**32**) was particularly complex due to the interconversion and chemical isomerisation of these isomers to linalool (**16**) under acid conditions. Thus, it is not unexpected that **21**, the main biotransformation product of linalool (**16**) by *B. cinerea*, also appeared in this experiment. In this case, the ω-hydroxylation of the substrate also predominates and occurs together with the reduction of the 6,7-double bond. The following compounds are the major transformation products in grape must: (2*Z*,6*E*)- and (2*E*,6*E*)-3,7-dimethylocta-2,6-dien-1,8-diol (**33** and **35**) and (*Z*)- and (*E*)-3,7-dimethyloct-2-en-1,8-diol (**34** and **36**), respectively, from nerol (**31**) and geraniol (**32**), along with some other minor products.



When the synthetic and the mixed media are used, the same metabolites are found, but in different quantities.

In a similar context, Schwab et al. studied the stereoselective formation of metabolites from 2-methylhept-2-en-6-one by *B. cinerea* [31]. This compound is another monoterpene that had been found in small quantities as a biotransformation product of some monoterpenic alcohols used as substrates by Bock et al. [24,25].

The compound obtained as the major biotransformation product was (*S*)-(+)-2-methylhept-2-en-6-ol, which once again demonstrates the reducing ability of *B. cinerea*.

Considering the different biotransformation products formed from monoterpenic substrates, it may

be concluded that *B. cinerea* has a considerable ω-hydroxylase activity and strong reducing power.

In addition, in 1988, Bock et al. [32] conducted a study on the biotransformation of cinnamaldehyde and various unsaturated acids, such as (*E*)-hex-3-enoic acid and (*E*)-oct-3-enoic acid, with four different strains of *B. cinerea* in two different culture media. These researchers were primarily interested in the reducing capacity of the fungus, which had already been observed in the bioconversion products of monoterpenic alcohols. In all the cases studied, microbial reduction to (*E*)-cinnamic alcohol and to dihydrocinnamic alcohol was observed, together with (*E*)-hex-3-en-1-ol and (*E*)-oct-3-en-1-ol, respectively, as the major compounds. Small quantities of other biotransformation products were also obtained, including (*Z*)-cinnamic alcohol, (*E*)-2-, (*Z*)-3-, and (*Z*)-hex-2-en-1-ol as well as (*E*)-2- and (*Z*)-oct-3-en-1-ol and oct-1-en-3-ol. These results clearly show that *B. cinerea* is capable of reducing certain compounds under favourable conditions.

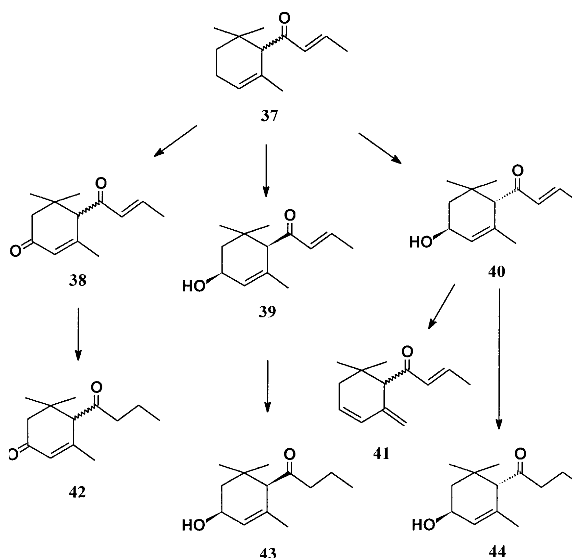
Similar observations had been reported previously, in 1987, by Urbasch [33], who used (*E*)-hex-2-enal as the substrate. The metabolisation of (*E*)-hex-2-enal, one of the main volatile components of plants with antibiotic action, was carried out with five different isolates of *B. cinerea*. Upon using isolates formed solely from mycelium, (*E*)-hex-2-enal was transformed into (*E*)-hex-2-en-1-ol. In contrast, when sporulating isolates were used, the substrate was hexan-1-ol. Essentially the metabolisation of (*E*)-hex-2-enal developed in the same way in both the liquid and the gas phases.

The products of the biotransformation of (*E*)-hex-2-enal were shown to be less fungitoxic against *B. cinerea* than the original substrate. Therefore, in this case, as in those discussed previously, the transformation reactions represent detoxification mechanisms by the fungus.

In 1989, some studies were published on the bioconversion of cyclic terpenoid ketones such as the damascones, which are the components of many natural aromas, e.g. α-damascone (**37**) has been described as a flavour constituent of black tea and tobacco [28]. The damascones generally have fruity and flowery aromas and are, thus, in high demand for the composition of perfumes, making them of considerable interest to industry. Hence, the microbial conversion of these compounds, a much cheaper process than chemical methods of synthesis, is very desirable goal.

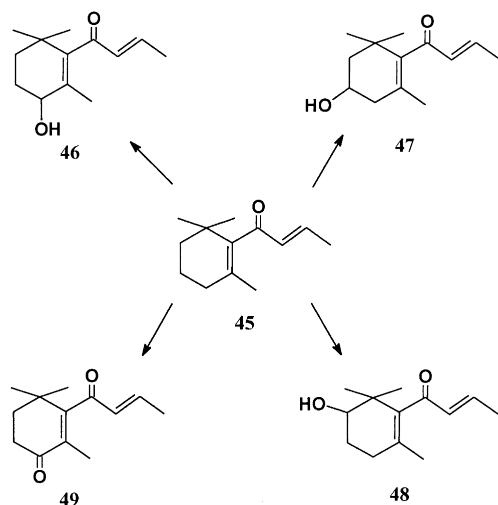
In this context, Schoch et al. [34] studied the biotransformation of α-damascone (**37**) by four particular strains of *B. cinerea* in grape must (pH 3.2). The transformation products obtained were 3-oxo-α-damascone (**38**), *cis*- and *trans*-3-hydroxy-α-damascone (**39** and **40**), γ-damascenone (**41**), 3-oxo-8,9-dihydro-damascone (**42**) and *cis*- and *trans*-3-hydroxy-8,9-dihydro-α-damascone (**43** and **44**).

After conducting a study over a longer time period, the maximum degree of formation of biotransformation products was found to occur after 9 days of incubation. The products oxygenated in position 3 were the first to form, whereas the hydrogenated products appeared after 8 days of incubation, with the maximum degree of formation occurring on the 13th day. In light of these observations, a metabolic pathway for the microbial transformation of α-damascone (**37**) was postulated.

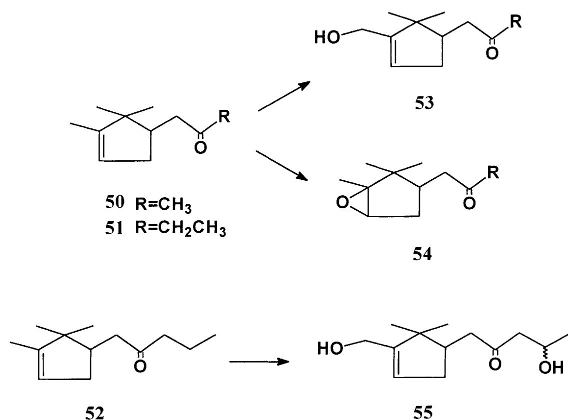


In view of the attractive aromatic properties of the damascones and given the considerable commercial interest in their synthesis, the same authors also studied the transformation of β-damascone (**45**) by *B. cinerea* [35] using both grape must and a mixture of must with synthetic medium. The results were found to depend quantitatively on both the medium and the strain used. With grape must as the culture medium, 4-hydroxy-β-damascone (**46**), 3-hydroxy-β-

damascone (**47**) and 2-hydroxy-β-damascone (**48**) were found to be the products of bioconversion. When the mixed medium was used, the above compounds were detected together with 4-oxo-β-damascone (**49**).

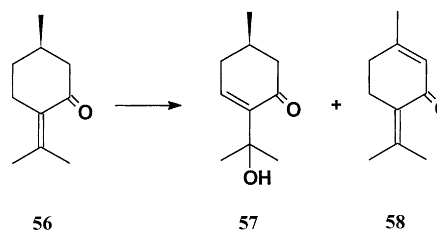


Earlier, in 1986, Dmochowska-Gladysz et al. [36] had studied the introduction of oxygen functions into isoprenoid systems by strains of *B. cinerea*. Methyl-, ethyl- and propyl-α-campholenone (**50**, **51** and **52**) were used as original substrates. The major products obtained from the first two substrates were the compounds resulting from hydroxylation in the position α with respect to the double bond (**53**), or the epoxidation of the double bond itself (**54**) (this latter product was found only in trace quantities). From propyl-α-campholenone (**52**) the only compound found was the product that had been hydroxylated in the position β with respect to the carbonyl group (**55**).



In 1991, Miyazawa et al. [37] carried out a series of studies on the biotransformation of (+)-pulegone (**56**) by *B. allii*. The animal conversion of this compound had already been studied in mice and rats, but there had been no work published on the microbial transformation. These authors added the product to a synthetic medium containing the spores of *B. allii* and obtained (−)-(1R)-8-hydroxymenth-4-en-3-one (**57**) as the major metabolite.

In the same year, these authors published another article [38] on the same bioconversion, making reference to the structure of the second main product, piperitenone (**58**), a compound characterised by its penetrating aroma of mint.



Also in 1991, Delfini et al. [39] studied the production of benzaldehyde and benzoic acid from benzyl alcohol by *B. cinerea* in synthetic medium, thus, demonstrating the oxidative capability of this fungus.

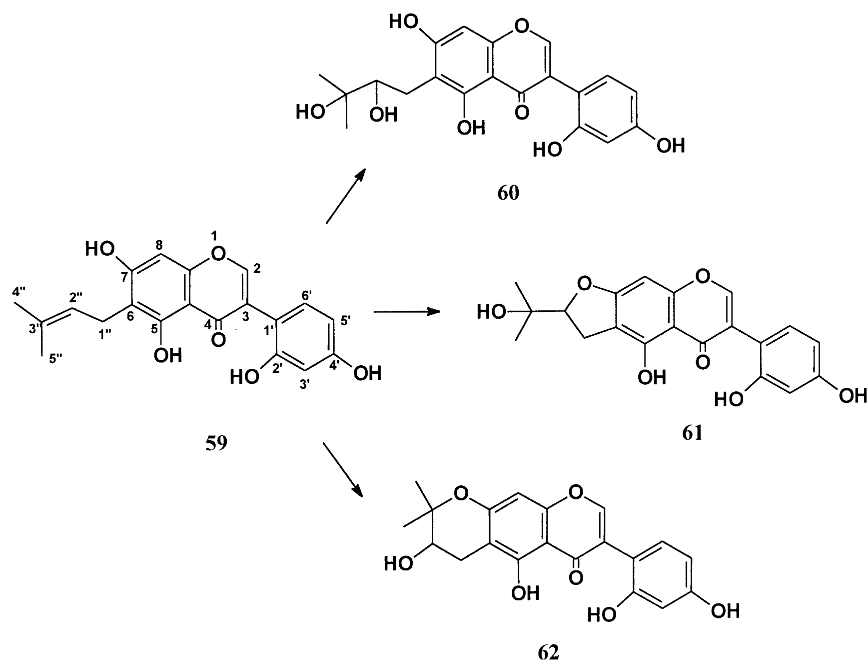
5. Biotransformations of antifungic flavones

Between 1984 and 1996, Tahara et al. [40,44–48,51, 53–55] followed a different line of research which dealt with the biotransformations produced by *B. cinerea* on flavones with antifungal activity.

In 1984, these authors studied the fungal transformation of luteone (**59**) [40], which is an isoflavone that was first isolated from the young fruits of *Lupinus luteus* L. This compound was found to possess significant antifungal activity, providing the plant with a degree of protection against potential fungal pathogens [41]. However, it is well known that some fungi are able to metabolise and detoxify isoflavonoid compounds, a fact that seems to be strongly related to their pathogenicity [42]. Because luteone (**59**) could act as a pre-infectional antifungal agent on leaves of *L. luteus* and other *Lupinus* species [43], it seemed useful to determine whether this isoflavone was susceptible to detoxification by microorganisms.

The bioconversion products obtained were principally 2″,3″-dihydrodihydroxyluteone (**60**), a dihydrofuranoisoflavone (**61**) and a dihydropyranoisoflavone (**62**), with the compound containing an epoxide between positions 2″ and 3″ proposed as an intermediate.

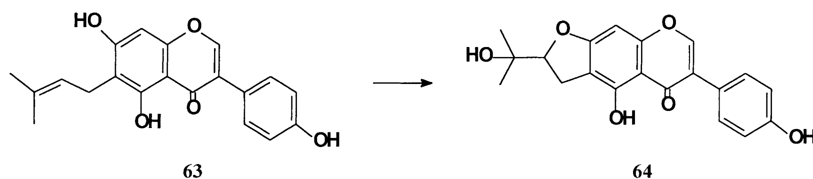
The metabolites obtained in the biotransformation were tested in antifungal activity bioassays and were found to be much less toxic than the substrate, which indicated that the biotransformation could have taken place as a detoxification mechanism of the fungus.



The derivative 7-methoxyluteone was also biotransformed by *B. cinerea* [44] (1989), giving the corresponding dihydropyranoisoflavone as the major metabolite along with a small quantity of dihydrofuranoisoflavone. At that time, no trace of the possible epoxide intermediate was detected in the biosynthesis of the substrate. However, in later studies by the same authors [45] (1993), this rather unstable intermediate in the biotransformation of 7-methoxyluteone was identified using the "resting cell" methodology. This epoxy-intermediate was easily cyclised under acid conditions and yielded the corresponding metabolites obtained in the biotransformation, thus, providing strong evidence for the author's theory.

In 1996, the same authors [46], using a cell-free system of *Botrytis cinerea*, showed that the transformation is initially catalysed by a microsomal monooxygenase, which gives rise to the epoxy intermediate. The microsomal reaction was carried out with 7-methoxyluteone as the substrate.

In 1985, Tahara et al. [47] studied the metabolites obtained from the biotransformation by *B. cinerea* of another antifungal isoflavone, wighteone (**63**). The principal metabolite obtained was the dihydrofuranoisoflavone **64**.



In the same year, these authors carried out identical research [48] with another prenylated isoflavone, licoisoflavone A. This compound is a fungitoxic regioisomer of luteone (**59**) in which the alkenyl side chain is situated at C3′ (B ring) rather than at C6 (A ring).

Although, licoisoflavone A was first isolated as a normal constituent of licorice (*Glycyrrhiza* sp.) roots [49], it was discovered that it accumulated as a phytoalexin in the fungus-infected pods of *Phaseolus vulgaris* [50]. Tahara et al. [51] discovered that considerable quantities (approximately 108 mg/kg fresh tissue) of this compound were accumulating in roots of the *Lupinus albus* plant (Leguminosae family), where together with luteone (**59**) and other isoflavones, it was able to retard or prevent the growth and development of attacking micro-organisms.

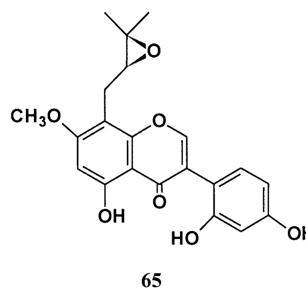
Licoisoflavone A was gradually metabolised after 3 days of incubation in a liquid medium containing a strain of *B. cinerea* and the transformation products obtained were a glycol (2′′,3′′-dihydrodihydroxylicoisoflavone A), two dihydrofuranoisoflavones and two hydroxydihydropyranoisoflavones, all with much less antifungal activity than the original substrate, once again demonstrating the detoxification mechanisms of the fungus.

The authors proposed that all of these biotransformations of prenylated isoflavones with *B. cinerea* take place by means of an epoxidation of the lateral chain in the original substrate, as mentioned above.

In contrast to the previously described isoflavones, the fungal metabolism of prenylated isoflavones at C8 had not been investigated due to their limited availability. However, roots of the *Lupinus luteus* plant were found to contain significant quantities of 2,3-dehydrokievitone [52], an isoflavone isolated earlier as a minor phytoalexin in other plants infected by fungi.

After incubation for 4 days in a shaken liquid medium containing an inoculum of *B. cinerea*, this isoflavone was metabolised [53] more slowly than its regioisomer, luteone (**59**). In this case, three main compounds were obtained: a dihydrofuranoisoflavone, a dihydropyranoisoflavone and a glycol, all of which were, in the opinion of the authors, derived from an intermediate epoxide.

In 1989, Tahara et al. [54] were finally able to obtain the epoxide that they considered to be the intermediate in all the biotransformations of prenylated isoflavones by *B. cinerea*. They methylated 2,3-dehydrokievitone with diazomethane to obtain the corresponding 7-methoxy derivative, which was metabolised by *B. cinerea* 10 times faster than the non-methylated compound to give the epoxide of 7-methoxy-2,3-dehydrokievitone (**65**) and the corresponding glycol. This effectively proved the existence of epoxide **65**, which serves as the intermediate in the biosynthesis of the bioconversion compounds of the isoflavones with a prenylic side chain.



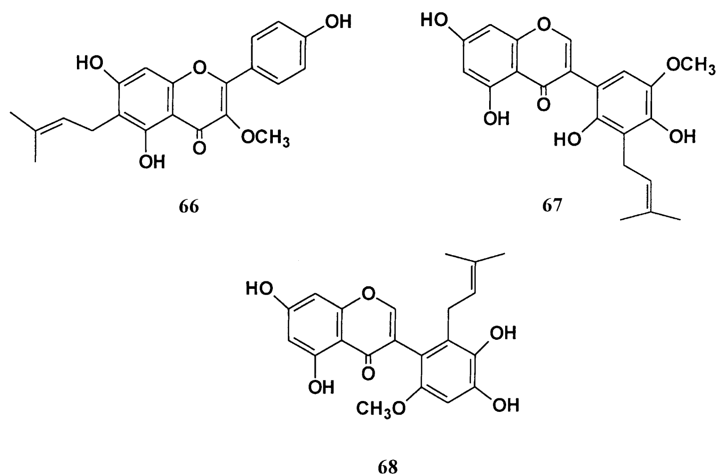
65

As a continuation of their research on prenylated flavonoids, in 1991, Tahara et al. [55] investigated new biotransformations by *B. cinerea*. Topazolin [5, 7, 4′-trihydroxy-3-methoxy-6-(3,3-dimethylallyl)-flavone] (**66**), piscerythrone [5,7,2′,4′-tetrahydroxy-5′-methoxy-3′-(3,3-dimethylallyl)isoflavone] (**67**) and piscidone [5,7,4′,5′-tetrahydroxy-2′-methoxy-6′-(3,3-dimethylallyl)isoflavone] (**68**) were metabolised and all gave a large number of products. The first two were converted into compounds similar to those obtained from luteone (**59**) and from licoisoflavone A, respectively, while piscidone (**68**) was metabolised to give the corresponding dihydropyranoisoflavone as the major product. Once again, the existence of an epoxide intermediate in the biosynthesis was demonstrated.

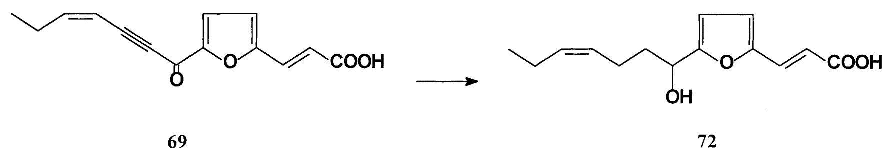
6. Biotransformations of phytoalexins and antifungal compounds

Between 1973 and 1976, a series of studies was published by Mansfield et al. [56–58] on possible detoxifications carried out by *B. cinerea* and *B. fabae*. These authors studied the metabolism of a series of phytoalexins induced by these fungi in the plant *Vicia faba* L.

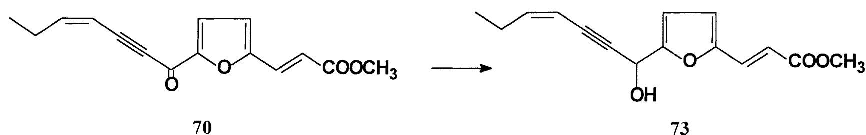
In this plant, both *B. cinerea* and *B. fabae* induced the formation of the phytoalexins wyeronic



acid (**69**), wyerone (**70**) and wyerone epoxide (**71**). The metabolism of **69**, which was published in 1973 [57], indicates that this phytoalexin is metabolised by *B. fabae* more rapidly than by *B. cinerea*. The disappearance of **69** caused by *B. fabae*, but not by the other species in question, was associated with the formation of a compound that was identified as reduced wyeronic acid (**72**), which is much less fungitoxic than the initial product. The concentration of **72** in seeds infected by *B. fabae* increased rapidly between the first and third day following inoculation and was not detected in the healthy tissues of the plant. In addition, extracellular enzymes were found to be incapable of degrading wyeronic acid (**69**) detected in the pods infected by *B. fabae*. This would suggest that the metabolism of this phytoalexin takes place inside the hyphae of the fungus.



It, thus, appears that after both *B. cinerea* and *B. fabae* have induced the production of **69** by the



plant *V. faba* L., the different capacities of the fungi for spreading lesions in the infected plant is the result of their different sensitivities to the phytoalexin. This sensitivity may or may not be directly related to the ability to metabolise wyeronic acid (**69**). Various measurements of the changes in the concentration of this acid in infected leaves suggest that *B. fabae* may be able to metabolise it in vivo and, thus, avoid its accumulation. The metabolism of **69** could, thus, turn out to be of tremendous significance in the pathogenicity of *B. fabae* towards bean plants.

In 1976, the same authors reported the in vitro conversion of the phytoalexin wyerone (**70**) into wyerol (**73**) by *B. cinerea* and *B. fabae* [56]. First, the phytoalexin **70** was induced to accumulate in cotyledons of the plant *V. faba* infected separately by *B. cinerea* and *B. fabae*. The compound, which is an acetylenic keto ester, was then transformed in vitro by both species of fungus into the corresponding hydroxyester, wyerol (**73**), which is less antifungal.

Hence, the conversion of **70** into **73** may be considered to be a detoxification mechanism of the

fungus. One interesting finding was that both compounds are more active against *B. cinerea* than against *B. fabae*.

The antifungal compound wyerone (**70**) was isolated for the first time from the seeds of broad bean plants germinated in darkness for 8 days, and was considered to be present only in healthy tissues of the plant *V. faba* L. [59]. Later studies concluded that this inhibitor was present only in trace levels in healthy tissues, that it accumulated after fungal infection as wyeronic acid (**69**), and that it should, therefore, be considered a phytoalexin [60].

The fact that there are no differences between the two species of *Botrytis* in their capacity to metabolise wyerone (**70**) suggests that their different sensitivity to the inhibitor could be due to some other mechanism apart from detoxification. This contrasts with the metabolism of wyeronic acid (**69**) carried out by these fungi, since the conidia of *B. fabae* but not those of *B. cinerea* were capable of metabolising **69** into the hexahydric derivative (**72**), which accumulated in the lesions caused by *B. fabae*.

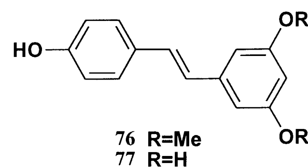
In addition to **69** and **70**, various antifungal compounds have been identified that are not present in healthy tissues of the plant, such as wyerone epoxide (**71**), the third acetylenic phytoalexin in *V. faba* [58]. Epoxide **71** accumulated in lesions produced by both *B. cinerea* and *B. fabae*.

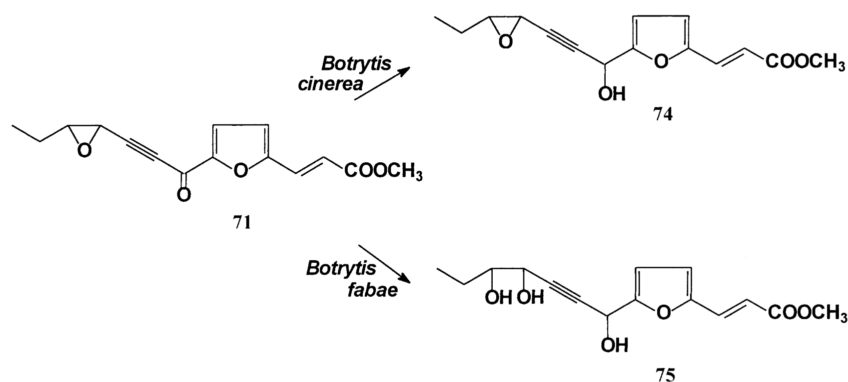
Products of the metabolism of wyerone epoxide (**71**) by both species of *Botrytis* were identified as wyerol epoxide (**74**) in the case of *B. cinerea* and as dihydrodihydroxywyerol (**75**) in the case of *B. fabae*. The metabolites obtained were found to be less antifungal than wyerone epoxide (**71**).

Compound **71** has been identified as a third component of the multiple phytoalexinic response of the plant *V. faba* to fungal infection by *Botrytis* [61]. The accumulation of epoxide **71**, which reaches antifungal concentrations in a limited number of lesions, indicates that it could play an important role in the inhibition of fungal growth in the infected tissues.

Wyerone epoxide (**71**) is more active than wyerone (**70**) against both species of *Botrytis*, but is a slightly less active inhibitor than wyeronic acid (**69**) under the conditions used for the bioassay. Epoxide **71**, as with other derivatives of wyerone (**70**) except wyerol epoxide (**74**), is more active against *B. cinerea* than against *B. fabae* [60]. Although, the major metabolite (**75**) obtained from phytoalexin **71** by *B. fabae* is less antifungal than that produced by *B. cinerea* (**74**), it is unlikely that the difference in sensitivity of these fungi to wyerone epoxide (**71**) is associated with the detoxification action. To date it has not been determined whether the above conversions would be produced by *Botrytis* in vivo.

On the subject of the detoxification of phytoalexins, of which *B. cinerea* is capable, an interesting article was published in 1991 by Pezet et al. [62]. These authors studied the oxidative detoxification of pterostilbene (**76**) and resveratrol (**77**) by a laccase-like stilbene oxidase produced by the fungus.





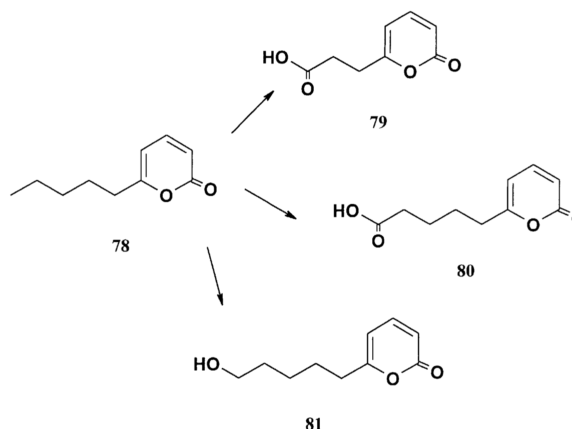
Pterostilbene (**76**) and resveratrol (**77**) are constituent components of the woody parts of many species of *Vitaceae* [63]. However, they are produced in the leaves and fruit only after fungal infection or exposure to UV radiation and may, therefore, be considered as phytoalexins. Some authors have suggested that both the speed and intensity of the formation of the stilbenic compounds indicate the plant's resistance to fungal infection.

If phytoalexins are important factors in the resistance of a plant to phytopathogenic fungi, then the capacity of a parasite to detoxify these compounds could be an important factor in the pathogenic mechanisms of parasites of that plant. *B. cinerea* is already known for its ability to metabolise and detoxify phytoalexins in a large number of plants. In the aforementioned study, the capacity of *B. cinerea* to oxidise pterostilbene (**76**) and resveratrol (**77**) and, thus, destroy their antifungal activity was investigated. The enzyme involved in these transformations was partially purified and characterised as stilbene oxidase, a compound belonging to the laccase-like enzyme family.

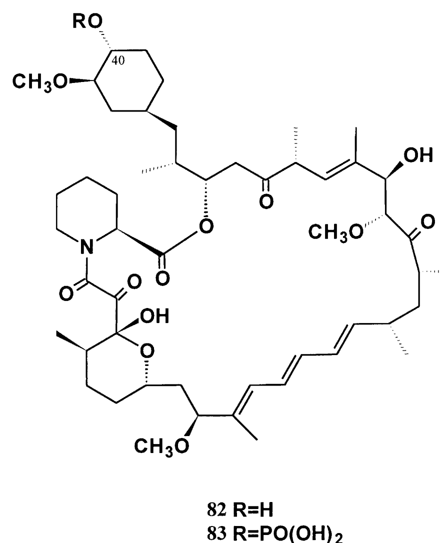
The crude protein solution was placed in contact with the substrates and after 10 min of action, 100% of the pterostilbene (**76**) and 94% of the resveratrol (**77**) had been degraded into a considerable number of non-fungitoxic compounds.

Sbaghi et al. [64] (1996) demonstrated that all the strains of *B. cinerea* that degraded these phytoalexins were highly pathogenic to in vitro cultures of grapevines (*Vitis rupestris*), while those strains that could not degrade phytoalexins were not pathogenic.

In 1997, Poole et al. [65,66] studied the biotransformation of 6-pentylpyr-2-one (**78**), an antifungal *Trichoderma* metabolite [66], by *B. cinerea* in liquid cultures [65]. Metabolism of **78** produced the compounds 3-(2-pyron-6-yl)propanoic acid (**79**), 5-(2-pyron-6-yl)pentanoic acid (**80**) and 5-(2-pyron-6-yl)pentan-2-ol (**81**). These metabolites were stable in the presence of actively growing *Botrytis cinerea* cultures and did not appear to alter fungal growth. It was proposed that the changes to the alkyl side chain in the metabolites reduced their lipophilicity and in turn decreased their ability to permeate cell membranes relative to the parent compound, thus, reducing their toxicity to the fungus.



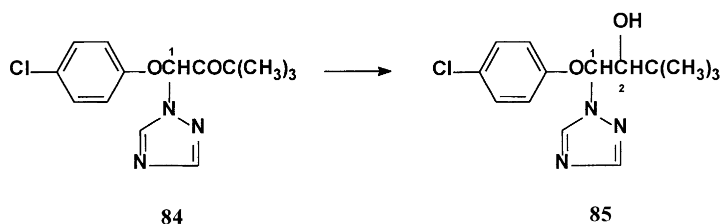
In the same year, Kuhnt et al. [67], with the aim of obtaining derivatives of rapamycin (**82**), an antibiotic with antifungal effect, screened a total of 28 bacterial and 72 fungal strains (as *Botrytis elliptica*) for their ability to transform this compound. The conversion product of rapamycin (**82**) isolated out of *Botrytis elliptica* was the 40-*o*-phosphoric ester of rapamycin (**83**).



As a special case, Deas et al. [68,69] conducted a series of parallel studies between 1984 and 1986 on the biotransformation carried out by *B. cinerea* on the fungicide triadimefon (**84**).

It had already been established that many species of fungi reduce the fungicide triadimefon (**84**) to triadimenol (**85**) [70–74]. These authors, thus, concentrated

on the study of the enantiomeric nature of the final compound, since triadimenol (**85**) possesses two chiral centres in C1 and C2, four enantiomeric forms should exist.



B. cinerea was grown in shake culture in a liquid nutrient medium containing different quantities of triadimefon (**84**) (4–40 mg/l). Triadimend (**85**) was the only biotransformation product obtained. Analysis by means of gas–liquid chromatography revealed the presence of (1*R*,2*S*)- and (1*S*,2*S*)-triadimenol, but neither of the (1*S*,2*R*)- or (1*R*,2*R*)-enantiomers was found. The proportion of the two diastereoisomers found decreased over a period of 6 to 168 h and with increased amounts of applied triadimefon (**84**). In other words, even though both (1*R*)- and (1*S*)-triadimefon were used as substrates for the reductase system of the fungus, the carbonyl group was specifically reduced to give only the alcohol with the *S*-configuration. These results may be explained in terms of the presence of: (a) an enzyme capable of accepting either (1*R*)- or (1*S*)-triadimefon as a substrate, but only yielding products of (2*S*)-configuration, or (b) two enzymes, one of which accepts the (1*R*)- and the other (1*S*)-isomer as substrate, but both yielding only (2*S*)-products. The second of these possibilities seems to be the more reasonable, and this is in agreement with the studies reported by Gasztonyi et al. [75] (1984), which concluded that more than one fungal enzyme is responsible for the reduction of **84** to **85**.

The fact that only certain stereoisomers are formed must be related to differences in fungitoxicity between these isomers [76]. Thus, it can be concluded that the stereoisomers found in the medium, i.e. (1*R*,2*S*) and (1*S*,2*S*) are the least active against *B. cinerea*.

It had previously been suggested [71] that triadimefon (**84**) itself could not be fungitoxic and that for its effectiveness as a fungicide it needed to be "activated" by reduction to triadimenol (**85**). From the results presented by Deas et al. [68,69], this possibility still remains unproven.

7. Conclusions

In summary, it is clear that the *Botrytis* species have a considerable hydroxylase and oxidase activity, mainly when these fungi metabolise phytoalexins [16,40,44–48,51,53–55,62] or antifungal compounds [18,21,22,65–67] as a detoxification mechanism.

Research has also shown that in some cases, these fungi are able to reduce antifungal compounds to give products with less toxic power [33,56–58,68,69], thus, indicating the significant ability of the *Botrytis* species to effect detoxification.

With compounds lacking fungitoxic activity (mainly aromas), the *Botrytis* species may behave both as an oxidising [4–7,19,20,23–26,34–36,39] and as a reducing [27,31,32] agent, depending on the substrate, however, the oxidising power is generally superior. In the case of monoterpenic substrates, *Botrytis cinerea* displays a considerable hydroxylase activity and strong reducing power.

Finally, it can be concluded that biotransformations by *Botrytis* species have a significant importance due to their broad utility for the enantiospecific production of compounds of commercial interest (perfumes, aromas), as well as for permitting the study of the metabolism of such compounds in order to obtain novel antifungal agents with activity against these micro-organisms.

References

[1] J.R. Coley-Smith, K. Verhoeff, W.R. Jarvis, The Biology of *Botrytis*, Academic Press, London, 1980, pp. 153–175.

[2] M. Wainwright, An Introduction to Fungal Biotechnology, Wiley, 1992.
[3] J.R. Hanson, An Introduction to Biotransformations in Organic Chemistry, W.H. Freeman (Ed.), Spektrum, 1995.
[4] D.S. Fukuda, D.R. Brannon, Appl. Microbiol. 21 (1971) 550.
[5] D.A. Dejong, A.D. King, F.P. Boyle, Am. J. Enol. Vitic. 19 (1968) 228.
[6] M. Shirasaka, Chem. Pharm. Bull. 9 (1961) 152.
[7] Ch.E. Holmlund, K.J. Sax, R.H. Blank, R.H. Evans, Steroids 5 (1965) 459.
[8] P. Flesch, L. Robbel, Z. Naturforsch 35C (1980) 88.
[9] A. Nespiak, A. Noculak-Palczewska, Z. Krolicki, J. Krzyzanowska, Acta Mycol. 19 (1983) 65.
[10] R. Croteau, Geruh und Geschmackstoffe, Verlag Hans Carl, Nürnberg, 1975, p. 153.
[11] R.P. Collins, A.F. Halim. J. Nat. Prod. 33 (1970) 481.
[12] A.F. Halim, R.P. Collins, J. Nat. Prod. 34 (1971) 451.
[13] R. Tressl, M. Apetz, R. Arrieta, K.G. Grünewald, Flavor of Foods and Beverages, Chemistry and Technology, Academic Press, New York, 1978, p. 145.
[14] G.L. Fagan, R.E. Kepner, A.D. Webb, Vitis 20 (1981) 36.
[15] T. Oritani, K. Yamashita, Agric. Biol. Chem. 44 (1980) 2637.
[16] E.W.B. Ward, A. Stoessl, Phytopathology 62 (1972) 1186.
[17] A. Stoessl, C.H. Unwin, E.W.B. Ward, Phytopathology 74 (1972) 141.
[18] I.G. Collado, J.R. Hanson, A.J. Macías-Sánchez, D. Mobbs, J. Nat. Prod. 61 (1998) 1348.
[19] R. Durán, E. Corrales, R. Hernández-Galán, I.G. Collado, J. Nat. Prod. 62 (1999) 41.
[20] R. Durán-Patrón, J. Aleu, R. Hernández-Galán, I.G. Collado, J. Nat. Prod. 63 (1999) 44.
[21] J. Aleu, J.R. Hanson, R. Hernóndez-Galán, I.G. Collado, J. Nat. Prod. 62 (1999) 437.
[22] J. Aleu, R. Hernóndez-Galán, J.R. Hanson, P.B. Hitchcock, I.G. Collado, J. Chem. Soc., Perkin Trans. I 6 (1999) 727.
[23] J. Shimizu, M. Uehara, M. Watanabe, Agric. Biol. Chem. 46 (1982) 1339.
[24] G. Bock, I. Benda, P. Schreier, J. Food Sci. 51 (1986) 659.
[25] G. Bock, I. Benda, P. Schreier, ACS Symp. Ser. 1986, 317 (Biogeneration of Aromas), pp. 243–253.
[26] P. Brunerie, I. Benda, G. Bock, P. Schreier, Appl. Microbiol. Biotechnol. 27 (1987) 6.
[27] P. Brunerie, I. Benda, G. Bock, P. Schreier, Z. Naturforsch 42C (1987) 1097.
[28] K. Bauer, D. Garbe, Common Fragance and Flavor Materials. Preparation, Properties and Uses, VCH Verlagsgesellschaft, Weinheim, 1985.
[29] G. Bock, I. Benda, P. Schreier, Appl. Microbiol. Biotechnol. 27 (1988) 351.
[30] P. Brunerie, I. Benda, G. Bock, P. Schreier, in: Bioflavour'87, Proceeding of the International Conference, 1988, p. 435.
[31] E. Schwab, A. Bernneuther, P. Puapoomchareon, K. Mori, P. Schreier, Tetrahedron: Asymmetry 2 (1991) 471.
[32] G. Bock, I. Benda, P. Schreier, Z. Lebensm Unters Forsch 186 (1988) 33.
[33] I. Urbasch, Z. Naturforsch 42C (1987) 64.
[34] E. Schoch, I. Benda, P. Schreier, Appl. Environ. Microbiol. 57 (1991) 15.
[35] E. Schwab, P. Schreier, I. Benda, Z. Naturforsch 46C (1991) 395.
[36] J. Dmochowska-Gladysz, T. Kolek, A. Siewinski, K. Derdzinski, A. Zabza, A. Nespiak, J. Basic Microbiol. 26 (1986) 577.
[37] M. Miyazawa, H. Huruno, H. Kameoka, Chem. Express 6 (1991) 479.
[38] M. Miyazawa, H. Huruno, H. Kameoka, Chem. Express 6 (1991) 873.
[39] C. Delfini, P. Gaia, L. Bardi, G. Mariscalco, M. Contiero, A. Pagliara, Vitis 30 (1991) 253.
[40] S. Tahara, S. Nakahara, J. Mizutani, J.L. Ingham, Agric. Biol. Chem. 48 (1984) 1471.
[41] H. Fukui, H. Egawa, K. Koshimizu, T. Mitsui, Agric. Biol. Chem. 37 (1973) 417.
[42] H.D. Van Etten, D.E. Matthews, D.A. Smith, in: J.A. Bailey, J.W. Mansfield (Eds.), Phytoalexins, London, 1982, p. 181.
[43] J.L. lngham, S. Tahara, J.B. Harborne, Z. Naturforsch 38C (1983) 194.
[44] S. Tahara, J.L. Ingham, J. Mizutani, Nippon Nögeikagaku Kaishi 63 (1989) 999.
[45] S. Tahara, F. Saitoh, J. Mizutani, Z. Naturforsch 48C (1993) 16.
[46] M. Tanaka, J. Mizutani, S. Tahara, Biosci. Biotechnol. Biochem. 60 (1996) 171.
[47] S. Tahara, S. Nakahara, J.L. Ingham, J. Mizutani, Nippon Nögeikagaku Kaishi 59 (1985) 1039.
[48] S. Tahara, S. Nakahara, J. Mizutani, J.L. Ingham, Agric. Biol. Chem. 49 (1985) 2605.
[49] T. Kinoshita, T. Saitoh, S. Shibata, Chem. Pharm. Bull. 26 (1978) 141.
[50] M.D. Woodward, Phytochemistry 18 (1979) 363.
[51] S. Tahara, J.L. lngham, S. Nakahara, J. Mizutani, J.B. Harborne, Phytochemistry 23 (1984) 1889.
[52] Y. Hashidoko, S. Tahara, J. Mizutani, Agric. Biol. Chem. 50 (1986) 1797.
[53] S. Tahara, E. Misumi, J. Mizutani, J.L. Ingham, Z. Naturforsch 42C (1987) 1055.
[54] S. Tahara, J.L. Ingham, J. Mizutani, Phytochemistry 28 (1989) 2079.
[55] S. Tahara, J.L. Ingham, J. Mizutani, Z. Naturforsch 46C (1991) 341.
[56] J.A. Hargreaves, J.W. Mansfield, D.T. Coxon, Phytochemistry 15 (1976) 651.
[57] J.W. Mansfield, D.A. Widdowson, Physiol. Plant Pathol. 3 (1973) 393.
[58] J.A. Hargreaves, J.W. Mansfield, D.T. Coxon, K.R. Price, Phytochemistry 15 (1976) 1119.
[59] C.H. Fawcett, D.M. Spencer, R.L. Wain, A.G. Fallis, E.R.H. Jones, M. Le Quan, C.B. Page, V. Thaller, D.C. Shubrook, P.M. Whitman, J. Chem. Soc. (C) 19 (1968) 2455.
[60] R.M. Letcher, D.A. Widdowson, B.J. Deverall, J.W. Mansfield, Phytochemistry 9 (1970) 249.
[61] J.A. Hargreaves, J.W. Mansfield, Ann. Appl. Biol. 81 (1975) 271.
[62] R. Pezet, V. Pont, K. Hoang-Van, Physiol. Mol. Plant Pathol. 39 (1991) 441.

[63] J.M. Hart, Ann. Rev. Phytopath. 19 (1981) 437.
[64] M. Sbaghi, P. Jeandet, R. Bessis, P. Leroux, Plant Pathol. 45 (1996) 139.
[65] P.R. Poole, G. Whitaker, J. Agric. Food Chem. 45 (1997) 249.
[66] J.M. Cooney, D.R. Lauren, P.R. Poole, G. Whitaker, J. Nat. Prod. 60 (1997) 1242.
[67] M. Kuhnt, F. Bitsch, M. Ponelle, T. Fehr, J.J. Sanglier, Enzyme Microb. Technol. 21 (1997) 405.
[68] A.H.B. Deas, T. Clark, G.A. Carter, Pestic. Sci. 15 (1984) 63–71.
[69] A.H.B. Deas, G.A. Carter, T. Clark, D.R. Clifford, C.S. James, Pestic. Biochem. Physiol. 26 (1986) 10.
[70] T. Clark, D.R. Clifford, A.H.B. Deas, P. Gendle, D.A.M. Watkins, Pestic. Sci. 9 (1978) 497.
[71] M. Gasztonyi, G. Josepovits, Pestic. Sci. 10 (1979) 57.
[72] M. Gasztonyi, Pestic. Sci. 12 (1981) 433.
[73] A.H.B. Deas, D.R. Clifford, Pestic. Biochem. Physiol. 17 (1982) 120.
[74] H. Buchenauer, F.Z. Grossmann, Z. Pflanzenkrankh. Pflanzenschutz 89 (1982) 309.
[75] M. Gasztonyi, G. Josepovits, Pestic. Sci. 15 (1984) 48.
[76] W. Krämer, K.H. Büchel, W. Draber, Pesticide Chemistry: Human Welfare and the Environment, Pergamon, Oxford, 1983, Chapter 1, p. 223.