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Synthesis and activity of grape wood phytotoxins and related compounds

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Summary. The synthesis of analogues and derivatives of two o-hydroxyphenylacetylenes, eutypine and sterehirsutinal, the main phytotoxins isolated from the culture medium of *Eutypa lata* and *Stereum hirsutum*, is reported. Two means of synthesis are described, based on cyclisation, oxidation, oxidative decarboxylation or reduction reactions, and producing o-hydroxyphenylacetylene or benzofuran derivatives. Some of these synthetic compounds were tested on grapevine callus in order to compare their toxicity with the natural analogues.

Key words: synthesis, o-hydroxyphenylacetylene, benzofuran, calli.

Introduction

Eutypiose and esca are two serious diseases of grape wood. They are responsible for considerable losses in yield and are one of the main causes of a shortened productive life of vineyards (Moller *et al.*, 1981; Munkwold *et al.*, 1994).

As an alternative to control with sodium arsenite, a highly efficient, but very toxic agent that was recently banned, it was decided to look for pathogenically active secondary fungal metabolites to combat this disease.

The ascomycete *Eutypa lata* is the causal agent of eutypiose (also known as "dead arm") and it also occurs in esca-infected wood. From the culture medium of this fungus, the main phytotoxic compound, eutypine, was isolated in our laboratory (Renaud *et al.*, 1989) .

Stereum hirsutum is a basidiomycete involved in the last stage of esca (Larignon *et al.*, 1997). The chemical structure of sterehirsutinal, the main toxin isolated from it, is very close to eutypine (Fig. 1; Dubin *et al.*, 2000). In addition to an aldehyde substituent that is probably responsible for its biological activity, sterehirsutinal contains two vinylacetylenic chains and two o-substituted hydroxy groups, which facilitate cylisation to a benzofuranic form.

Several biogenetically related analogues of these two toxins were also isolated from the culture media, but not in sufficient amount to carry out a large array of biological activity tests. The synthesis of these compounds is nevertheless important in order to establish the toxicity of the different secondary metabolites, and to investigate the detoxification process by specific enzymes or micro-organisms (Christen *et al.*, 2003).

Results and discussion

The isolated analogues were essentially o-hydroxyphenylacetylene and benzofuran-substituted compounds. All can be considered derivatives of eu-

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typine. Indeed the occurrence of the hydroxy group in the ortho position on the acetylenic chain leads easily to a benzofuran derivative by cyclisation.

We followed two ways to synthesise all the compounds. The structures were confirmed by spectroscopic analysis and compared with the natural analogues. The cyclisation of **1** (Renaud *et al.*, 1989, Zesiger, 1991) was very easy. Treatment with Cu_2O (Fig.2) in pyridine led to the cyclisized product.

Oxidation or reduction reactions of eutypine (Defrancq *et al*, 1993) and of the benzofuran derivative **3** gave **4, 5, 6** (eutypine) and **7, 8 ,9** (benzofuran) with good yields.

Similar reactions are also possible starting from sterehirsutinal (Fig. 3), but this is more difficult because the synthesis of this toxin (Fkyerat *et al*, 1999) is tricky. The yield of the overall synthesis was low so that and we had only a small amount of material to work with. We noticed that treatment with Cu_2O in pyridine was needed for benzofuran formation. **2**, **11** and **12** led to a double cyclisation due to the presence of two vinyl-acetylenic chains in the ortho position of each hydroxy function. Dibenzofuran **10** has been isolated and characterised; it was found to be less stable than **3** at room temperature (Perrin-Cherioux *et al.*, 2003)

To compare the phytotoxicity of the synthetic derivatives with eutypine and sterehirsutinal we

1 Eutypine **2** Sterehirsutinal

Fig. 1 Chemical structure of eutypine and sterehirsutinal.

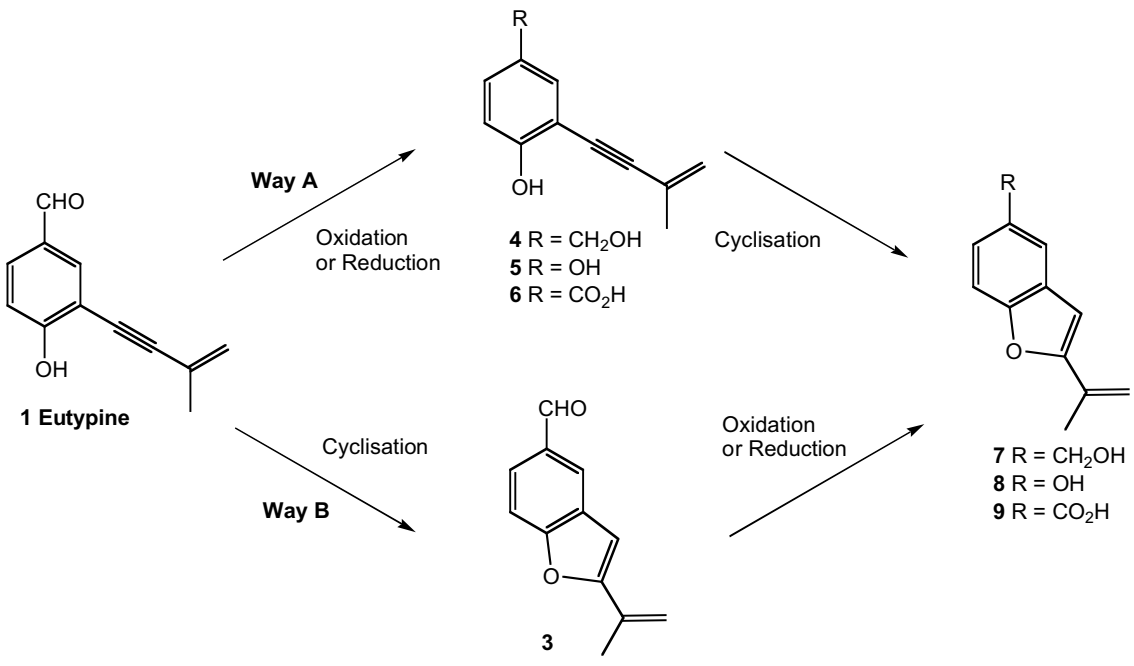

Fig. 2. Synthesis of eutypine derivatives.

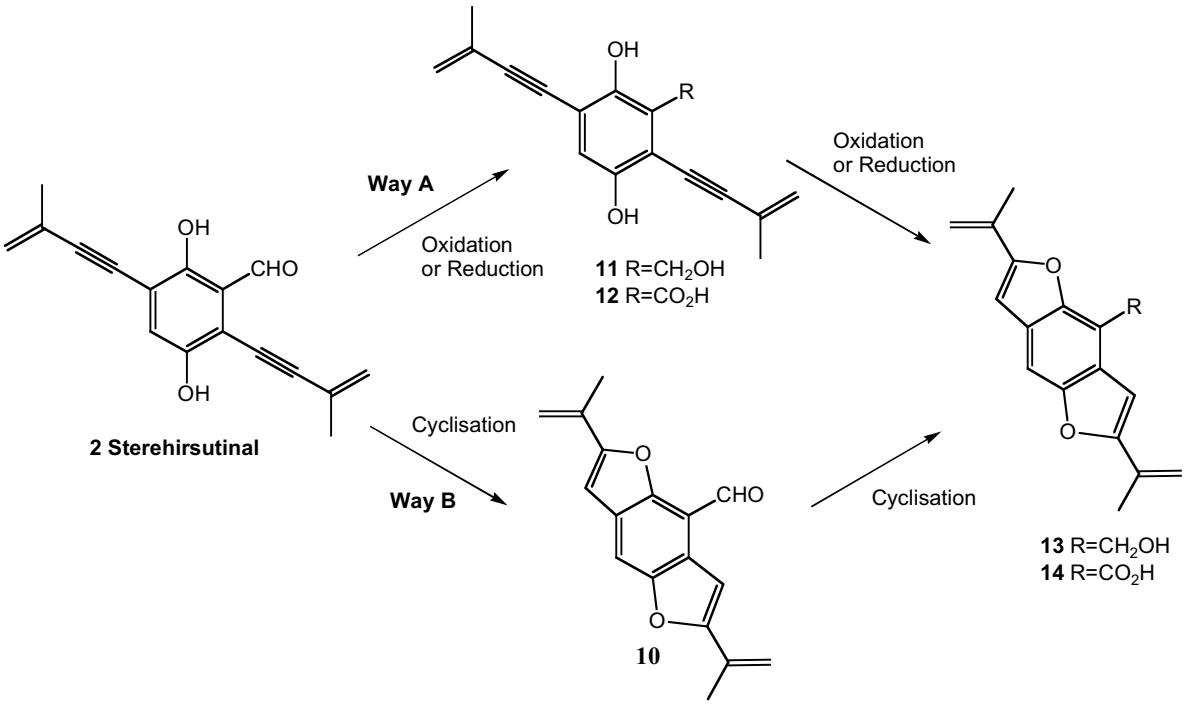

Fig. 3. Synthesis of sterehirsutinal derivatives.

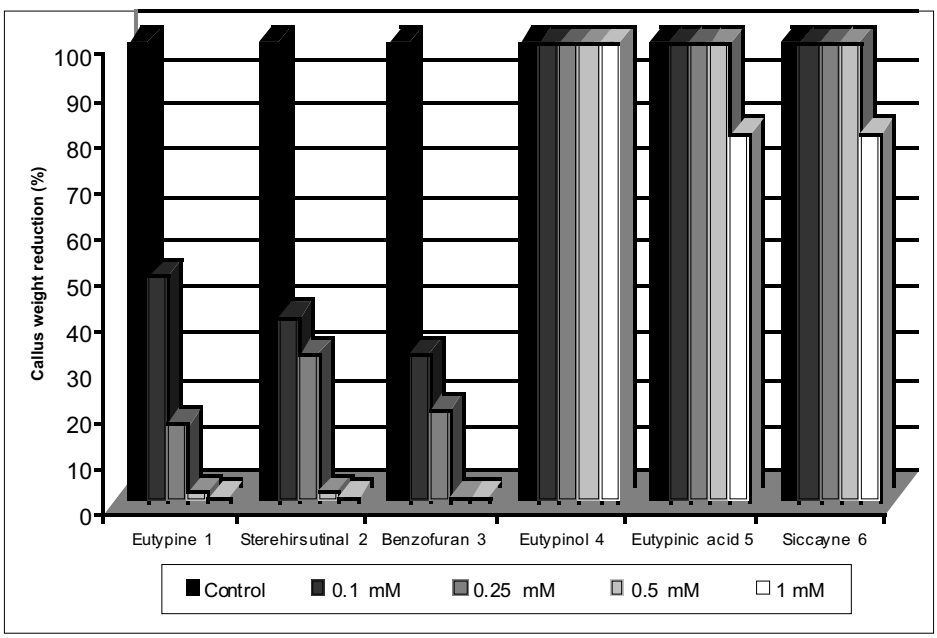

Fig. 4. Growth inhibition of Gamay callus by the synthetic eutypine derivatives compared with eutypine and sterehirsutinal.

carried out a simple biossay based on the growth inhibition of Gamay callus. These bioassays (Fig. 4) showed that eutypinol (**4**), eutypinic acid (**5**) and siccayne (**6**) were not phytotoxic to grape callus *in vitro*, but that they seemed to be growth promoters at concentrations lower than 0.1 mM. Eutypine (**1**) sterehirsutinal (**2**) and the benzofuranic form of eutypine (**3**) strongly inhibited growth. Similar results were observed with the derivative of sterehirsutinal (**10-14)** but quantification was not possible because of the small amount of available material.

The presence of other metabolites in the culture medium of *E. lata* and *S. hirsutum* suggested that eutypine and sterehirsutinal were probably not the only agents of phytotoxicity. Mahoney *et al* (2003), recently confirmed the phytoxoxicity of other metabolites from different strains of *E. lata*. The diseases caused may have resulted from several compounds produced by the fungus, and a synergistic effect was also possible.

In the infected vegetable material (branches, leaves, inflorescences, necrotised wood) only eutypine and sterehirsutinal were found in our laboratory and identified by tandem mass spectrometry (They-Ruhl, 1991; Dubin, 1998). *In vivo* eutypine can be reduced, in 'Merlot', by an NADPH-dependent aldehyde reductase (ERE) to the non-toxic eutypinol (Colrat *et al.,* 1999a, b). Detoxification by micro-organisms or specific enzymes is being investigated (Christen *et al.*, 2003).

Conclusions

Two synthetic pathways were developed starting from eutypine (**1**) and sterehirsutinal (**2**), by oxidation, oxidative decarboxylation, reduction (reactions frequently observed in biological systems) and cyclisation, yielding to potential bioactive derivatives. Chemical cyclisation of all compounds was performed because the benzofuran derivative might play a crucial role in the resistance or detoxification process.

The toxicity of sterehirsutinal, eutypine and its cyclisation products was confirmed on grapevine callus.

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