Pure & Appl. Chem., Vol. 66, Nos 10/11, pp. 2299–2302, 1994.
Printed in Great Britain.

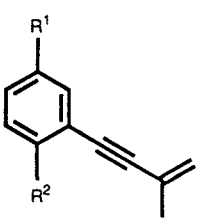
Secondary phytotoxic metabolites from pathogenic fungi: Structure, synthesis and activity

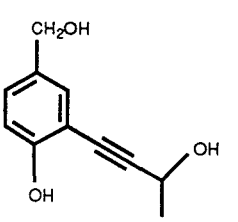
Raffaele Tabacchi
Institute of Chemistry, University of Neuchâtel
Av. de Bellevaux 51, CH-2000-Neuchâtel; Switzerland

Abstract
The secondary metabolites from three pathogenic fungii, *Eutypa lata, Ceratocistis fimbriata* var. *coffea* and *Ceratocistis fimbriata* var. *platani*, responsible respectively for the vine (*Vitis vinifera*), coffee tree (*Coffea* sp). and plane tree (*Platanus acerifolia* and *P. orientalis*,) diseases have been investigate. Eutypine (1) and other substituted phenylacetylenic compounds (2 -7) have shown a strong phytotoxic activity on the vine.The isocourmarins (12 - 16) seemed to play an important role into the canker diseases. The resistance factors to *Botrytis cinera* in grapevine have also been studied and six phenolic derivatives (22 - 28) that inhibiting stilbene oxydase have been isolated.

Introduction
Fungal diseases in plants are continually increasing. The biological and pathological aspects of these diseases are generally well known. In contrast, their chemical aspects and particulary the structure of the toxins, their role, and the defense mechanism of the plant have received little attention.
Four examples will be discussed.

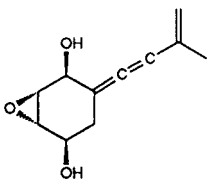
Results and Discussion
The ascomycete *Eutypa lata,* responsible for the dying arm disease of the grapevine, is currently causing an important economic problem throughout the world.
A family of phenylacetylenic compounds 1 - 7 has been isolated from the culture media of *Eutypa lata,* and was identified by chemical and spectroscopic techniques (1). One compound,-Eutypine (= Hydroxy-3-(3-methylbut-3-en-1-ynyl)benzaldehyde) showed extreme toxicity to the vine. Using tandem MS methods, we confirmed the presence of the toxin in the branches, the leaves, the inflorescences and the rising sap of vine. Eutypine (1), the 14-C labelled analogue and all the other derivatives have been synthesised (2). A series of novel epoxycyclohexane 8 - 11 and other biogenetically related compounds have also been isolated and synthesised (3). Biotests have shown an antibiotic and antimitotic activity.



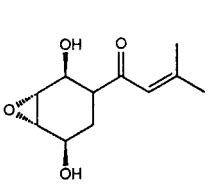


1 R^1 = CHO, R^2 = OH
2 R^1 = CHO, R^2 MEeO
3 R^1 = COOH, R^2 = OH
4 R^1 = R^2 = OH
5 R^1 = CH_2OH, R^2 = OH
6 R^1 = CH_2OH, R^2 = MeO

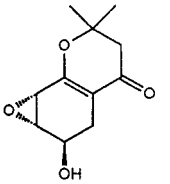
7



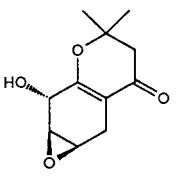
8



9

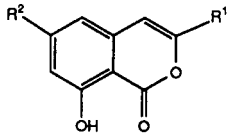


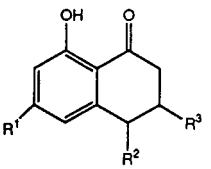
10



11

Ceratocistis fimbriata is a perithecial ascomycete which infects highly commercially valued crops and trees (coffee, cocoa, hevea, plane oak). Different strains have shown general specifity to their host. This pathogens enters through the wounds, into the roots and the branches, and causes foliar and fruit withering accompanied by trunk canker in adult trees (4). The strain we investigated was isolated in Colombia from the coffee tree and cultured on a synthetic medium in our laboratory. The ether extract of this medium has shown a toxic activity on coffee tree leaves (necrosis). Investigation on the toxic fractions has led to the isolation of five major metabolites. All the isolated compounds 12 - 16 belong to the isocoumarin class, which is known for it's inhibitory activity on plant growth. (5) We carried out a simple bio-assay based on necrosis induction. All the isolated isocoumarins, except 16 have tested positive.
Investigation of the less active part of the extract led to the isolation of four tetralone 17 - 20 derivatives which showed low activity to coffee tree leaves.





12 $R^1 = CH_2\text{-}CHOH\text{-}CH_3$, $R^2 = OH$

13 $R^1 = CH_2OH$, $R^2 = OCH_3$

14 $R^1 = CH_2OH$, $R^2 = OH$

15 $R^1 = CH_3$, $R^2 = OCH_3$

16 $R^1 = CH_3$, $R^2 = OH$

17 $R = R^1 = H$, $R2 = OH$

18 $R = R^2 = OH$, $R^1 = H$

19 $R = R^1 = R^2 = OH$

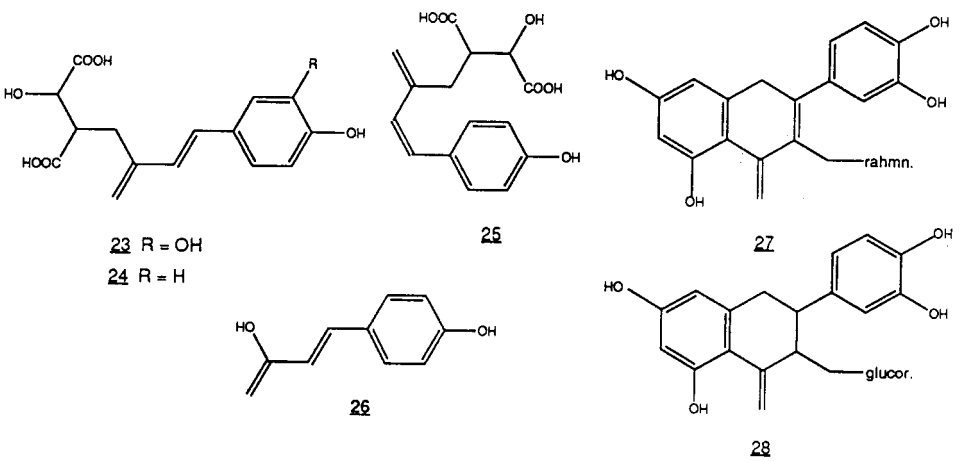
20 $R = H$, $R^1 = R^2 = OH$

21 $R = R^2 = H$, $R^1 = OH$

The plane tree (*Platanus acerifolia* and *Platanus orientalis*) is the most important urban tree in many towns of Italy, France, and southern Switzerland (6). It is strongly threatened by a lethal disease caused by the fungus *Ceratocistis fimbriata* sp. *platani*. This fungus, whose occurence was first reported in the USA, causes foliar withering, necrosis and canker leading to death of the tree. Preliminary studies have shown the extreme pathogenicity of this fungus inoculated on plane tree's healthy stems. Symptoms of the disease have been largerly described and the use of chemicals through systematic fungicides have not worked, and only prophylactic treatments have prevented the progression of the parasite from the French Midi and Italy to the North. Efficient prevention will be the creation of resistant clones. On the other hand, the chemical aspect of the disease, particularly the nature of the secondary metabolites, their phytotoxicity and the host-parasite interaction are totally unknown. The isolation and characterisation of the toxins is of primary importance. From a culture medium of a strain of *Ceratocistis fimbriata* sp. *platani*, isolated in south Switzerland, we investigated some fractions of the ether extract showing phytotoxic effects on plane leaves from *in vitro* culture. Further tests on plane callus are in progress. The major metabolites isolated have been identified as three teralones 18 20 and 21 and a monoterpene acid,(3S)-3 hydroxy-3,7 dimethyl-6 octenoic acid. (22) None of these compounds were very active in our bioassay. In

contrast from two other fractions, we isolated small amounts of tree isocoumarins that seems to be phytotoxic. The structures are not yet well established.

The gray mould, caused by the fungus *Botrytis cinerea*, is one of the most important disease occuring in the vineyards. Before blooming time and after veraison grape berries are sensitive to this parasite. Depending on the climate it can also provoke an economically important degradation of the berries. Between these two periods of development (called quiescent stage) grape berries are resistant to *Botrytis cinerea* which could be present inside them without parasitic and visible damage (7). Pezet and Pont (1986) showed that crude extracts of apparently healthy berries collected at this stage strongly inhibited germination of *B. cinerea* conidies (8) These extracts contained pterostilben (trans-3,5-dimethoxy-4-hydroxystilben), described earlier as a fungicidal phytoalexin produced by *Vitis vinifera* . However, the natural concentration of pterostilben detected in berries is too low to explain the biological activity of the crude extracts. Organic acids (glycolic, tartaric and malic acids) responsible for the low pH of grape berries associated with low concentration of pterostilben lethally inhibited germination of *B. cinerea* conidies and can explain the crude extract's fungicidal activity. It appears later that glycolic acid enhanced alone the biocidal activity of pterostilben . However, other chemical and mechanical process are known to be involved in maintaining the quiescent stage of *B. cinerea* : a glycoprotein inhibit exogenous polygalacturonase of the parasite as polyphenolic grape's tanins for fungal polyphenoloxidase. The epidermical complex can appear as a mechanical barrier to the development of the fungus (8,9). Other phenolic compounds are recognised as growth inhibitors. Consequently, the resistance process of the young berries is the result of multiple parameters, not yet entirely described. We discuss the identification of phenolic metabolites in grape berries which could be involved directly as precursors of fungicidal products responsible for the quiescent stage. For this purpose, free phenolic compounds 23 - 28 were separated from berries at different development stages, purified and identified by spectroscopical means without any chemical modifications.



The HPLC chromatograms of the free phenolic compounds extracted from clusters sensitive to *Botrytis cinerea* before flowering in June and from berries collected in the end of July during the natural resistant period have shown that some compounds were presents in both periods whereas during the sensitive period other peaks have appeared.

In order to compare quantitatively the content of these compounds between two grape varieties with different sensitivity to grey mould, we have analysed these phenolic extracts from *Gamay* and *Gamaret*. The first variety allows a high development of gray mould after veraison when climatic conditions are favourable and the second is considered as resistant to *Botrytis cinerea* in these conditions. The interesting compounds were isolated by semi-preparative HPLC and the structures elucidated. ^{1}H and ^{13}C NMR experiments at low temperature (-40°) and mass spectrometry data showed compounds 23 to be trans-caffeoyl tartaric acid.(trans-caftaric acid), 24: trans p-coumaroyl-tartaric acid (trans-coutaric acid), 25: cis -p-coumaroyl tartaric acid (cis-coutaric acid), 26: coumaric acid, 27: quercetin-3-O-rhamnosyde, and 28: taxifolin-3-O-glucoronate. All these compounds had been described in the grapes, however the appearence of compounds 23 and 24 together during the quiescent stage is demonstrated for the first time. Biotests (11) have confirmed that all the isolated compounds inhibit the stilbene oxydase at low concentration (0.002 - 0.2mM)
Structures elucidation of three other interesting compounds are presently in progress.

Acknowledgements
We would like to thank Drs. *J.-M. Renaud, P. de Angelis, G. Tsoupras, S. Claude, J. Gordon, E. Defrancq, T. Zesiger, M. Dai, N. Metais,* Mr. *G. Gremaud* and *N. Bürki,* for their cooperation. The *Swiss National Science Foundation* (grant no. 20-28922-90 and 20- 33936. 1992) is gratefully aknowledged.

References

1. J.- M. Renaud, G. Tsoupras and R. Tabacchi, *Helv. Chim. Acta* **72,** 929 (1979).
2. E. Defrancq, T. Zesiger and R. Tabacchi, *Helv. Chim. Acta* **76**, 425 (1993)
3. E. Defrancq, J. Gordon A. Brodard and R. Tabacchi, *Helv. Chim. Acta* **75**, 425 (1992); J. Gordon and R. Tabacchi, J. Org. Chem. **57**,4728 (1992).
4. R. E. Pontis, *Phytopathology* **41**, 178, 1951
5. G.W.McGraw and R. W. Hemingway, *Phytochemistry* **16**, 1315 (1977)
6 J.P. Ferrari and M. Pichenot *C.R. Acad. Sci.Paris* **258**, 2787 (1974); A. Vigouroux, *Rev, For. Fr.* 31,**28**, (1979);C. Gessler and C. Mauri, *Botanica Helv.* **97** 349, (1987)
7. R. Pezet and V. Pont, *Revue Suisse Vitic. Arboric* **18,** 317 (1986)
8. P. Jeandet and R. Bessis *Bulletin Office International Vigne et Vin* **703 - 704** 638(1989)
9. R. Pezet and V. Pont, *Phytopath. Z.* **111**, 73 (1988)
10. R. Pezet and V. Pont, *Plant Physiol. Biochem*, **26**, 603 (1988)
11. R. Pezet, V. Pont and K. Hong-Van, *Physiological and Molecular Plant Pathology* **39,** 441 (1991)