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STEROL COMPOSITION OF THE WOODY PLANT PATHOGENIC FUNGUS *EUTYPA LATA*

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Abstract—Mycelium of *Eutypa lata* grown in solid and liquid cultures contained C_{28}-sterols, mainly ergosterol, accompanied by much smaller amounts of episterol, ergostatetraenol, ergosta-7,22-dien-3β-ol and minor 4α-methyl and 4,4-dimethylsterols. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Eutypa dieback of grapevines (*Vitis vinifera* L.) is one of the most destructive diseases of its woody tissues and is caused by the ascomycetous fungus *Eutypa lata* (Pers.). This serious disease, which is equally prevalent in the United States, Australia and Europe, is difficult to control [1]. It is a significant cause of economic loss, and much effort has been devoted to developing effective fungicides for protecting vineyards against this fungus. Wound protection with benzimidazole fungicides has proved relatively successful [2] and inhibitors of the sterol biosynthesis have recently been used in the field on grapevines [3].

Whereas data is already available on the sterol composition of phytopathogenic fungi such as *Botrytis cinerea* [4], *Pyrenophora teres* [4], *Ustilago maydis* [5], *Fusarium* species [6], or powdery mildew [7, 8], no report has been published on the sterol composition of *Eutypa lata*.

In order to improve understanding of the biochemistry of *E. lata*, the present paper describes the sterols in the mycelium of *E. lata* grown in solid and liquid cultures.

RESULTS AND DISCUSSION

The percentage sterol composition and total sterol content of *Eutypa lata* are listed in Table 1. The sterols were identified as their acetate derivatives by comparison of mass spectra with the published data [4, 6, 9, 10]. The major sterol was identified as ergosterol (**2**); this 4-desmethylsterol accounted for 88% of total sterols in solid medium and 78% in liquid medium. In addition to ergosterol, four minor 4-desmethylsterols (ergosta-5,7,9(11),22-tetraen-3β-ol (**1**), ergosta-7,22-dien-3β-ol (**3**), fecosterol (**4**) and episterol (**5**)) were present and each accounted for between 1 and 4% of total sterols in solid medium and 1.8 and 13% in liquid medium. The major sterol difference noted between the media was the amount of episterol (**5**). This was three

Table 1. Sterol composition of *E. lata*

Sterols	RR_t^*	Sterols in media % of total Solid	Liquid
Ergostatetraenol (**1**)	1.135	2.52	3.11
Ergosterol (**2**)	1.176	88.06	78.60
Ergosta-7,22-dien-3βol (**3**)	1.183	2.66	1.80
Fecosterol (**4**)	1.204	1.14	1.88
Episterol (**5**)	1.228	4.32	13.13
4α-Methylfecosterol (**6**)	1.242	0.27	0.48
Lanosterol (**7**)	1.273	0.08	0.09
Eburicol (**8**)	1.304	0.46	0.47
4,4-Dimethylfecosterol (**9**)	1.318	0.22	0.23
Other sterols		0.27	0.22
4-Desmethylsterols		98.86	98.65
4α-Methylsterols		0.38	0.55
4,4-Dimethylsterols		0.76	0.79
Total amount of sterols (μg mg^{-1} dry wt)		1.58	1.12

*Relative retention time of steryl acetate relative to cholesterol.

All experiments were repeated at least twice with no less than two replicates and the s.e. never exceeded 5%.

Trivial names used: eburicol, (**8**), 4,4,14α-trimethylergosta-8,24(24^1)-dien-3β-ol; ergosterol (**1**), ergosta-5,7,22-trien-3β-ol; episterol (**5**), ergosta-7,24(24^1)-dien-3β-ol; fecosterol (**4**), ergosta-8,24(24^1)-dien-3β-ol; lanosterol (**7**), 4,4,14α-trimethylcholesta-8,24-dien-3β-ol; 4,4-dimethylfecosterol (**9**), 4,4-dimethylergosta-8,24(24^1)-dien-3β-ol; 4α-methylfecosterol (**6**), 4α-methylergosta-8,24(24^1)-dien-3β-ol.

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times more important in liquid medium than in solid medium. Two Δ^7-sterols were found with different unsaturation patterns on the side chain and identified as ergosta-7,22-dien-3β-ol (**3**) and episterol (**5**). Traces of an unidentified 4-desmethylsterol with relative retention time of 1.127 was also detected. The mass spectrum suggested that this was an isomer of ergosterol with *m/z* (rel. int): 438 $[M]^+$ (1), 378 $[M - Ac]^+$ (100), 363 $[M - Ac - Me]^+$ (23), 337 $[M - Ac - 42 + H]^+$ (4), 253 $[M - SC - Ac]^+$ (92), 211 $[M - SC - 42 - Ac]^+$ (35), intense peaks at *m/z* 157(69) and 143(42). Small amounts (1%) of fecosterol (**4**) were present.

The amounts of 4,4-dimethyl- and 4α-methylsterols were small (0.89 and 0.60% of total sterol, respectively). Trace amounts of sterols detected in *E. lata* included C-4 methylated sterols. Sterols **6** and **9** had similar mass spectra and were identified as 4α-methylfecosterol and 4,4-dimethylfecosterol, respectively.

Sterols **7** and **8** eluted in the 4,4-dimethylsterol fraction were identified as lanosterol and eburicol, respectively. Traces of ergostatetraen-3β-ol with 8(9)- and 14(15) double bonds and 4α-methylergosta-8,14,24(24^1)-trien-3β-ol were also detected, by GC-MS only. The mass spectrum of the ergostatetraen-3β-ol displayed a molecular ion peak at *m/z* 436 (18), a base peak at *m/z* 361 $[M - Ac - Me]^+$ and a peak at *m/z* 235 $[M - SC - Ac - Me - H]^+$ (25), suggesting the presence of a $\Delta^{8,14}$-diene [9]. The fragments at *m/z* 250 $[M - SC - Ac - H]^+$ (16) and 209 $[M - SC - C_3H_6 - Ac]^+$ (C_3H_6, loss of C-15 to C-17) suggested that three unsaturation sites were present in the steroid rings and one unsaturation site on the side chain. The putative structure may be an ergosta-5,8,14,22-tretraen-3β-ol as suggested by Debieu *et al.* [6]. The 4α-methylsterol with $\Delta^{8,14}$-unsaturations had a molecular ion peak at *m/z* 452 (92%), a base peak at *m/z* 353 $[M - 84 - Me]^+$ and showed the characteristic fragmentation pattern at *m/z* 326 $[M - SC - H]^+$ (39) and at 251 $[M - SC - Ac - Me - H]^+$ (33) of $\Delta^{8,14}$-sterols [9]. In addition, this compound had an olefinic bond in the side chain shown by *m/z* 353 and was identified as 4α-methylergosta, 8,14,24 (24^1)-trien-3β-ol [9, 11].

As with most ascomycetes, *E. lata* produces ergosterol as a major sterol [12] accompanied by several other C_{28}-sterols. In its sterol biosynthetic pathway, *E. lata* resembled other filamentous fungi in that lanosterol (rather than zymosterol as in yeasts) seemed to be the substrate used for introducing a methyl group in the side chain [13]. The presence of eburicol, a 4,4,14α-trimethylsterol unsaturated at C-24 suggests that alkylation at C-24 occurs prior to C-14 and C-4 demethylation as in most fungi except yeasts [14,15].

Lichesterol (ergosta-5,8,22-trien-3β-ol), a sterol commonly found in *Botrytis cinerea* [4], *Rhynchosporium secalis* [16], *Pyrenophora teres* [4], *Pyricularia oryzae* [17], and *Fusarium* species [6], was not detected in *Eutypa lata*. The ergostatetraenol present in *E. lata*, and which is also present in *Botrytis cinerea*, *Pyrenophora teres* [4] and *Fusarium* species, was identified as ergosta-5,7,9(11),22-trien-3β-ol and it could be derived from ergosterol as suggested by Atherton *et al.*, [18].

Our work suggests that the sterol biosynthesis pathway of *E. lata* is very similar to that of other pathogenic filamentous fungi, excluding the obligate pathogenic fungi such as powdery mildews [7, 8]. Plant protection fungicides, including triazoles have been demonstrated to inhibit the biosynthesis of ergosterol [19] and seemed effective against *E. lata* [3].

EXPERIMENTAL

Cultures of *Eutypa lata* (strain 8D from Provence) were maintained on malt-agar medium in Petri dishes. Mycelium explants (one plug with a 5 mm diameter) were incubated in 100 ml of liquid inorganic medium [20] for 10 days at 25°C under agitation. Harvested mycelium was freeze-dried and weighed.

Sterol extraction and analysis. Dried mycelium was saponified in methanolic KOH (6%) under reflux for 2 hr. The unsaponifiable lipids were extracted with hexane and sterols purified by silica gel TLC [21]. After acetylation, samples were injected (0.5 μl) via an on-column injector onto a 25 m × 0.32 mm i.d. OV-1 silica capillary column (H_2, 80 kPa carrier gas, FID detection). The oven temp. was increased from 60° to 230° at 10° min^{-1} and programmed 230° to 300° at 3° min^{-1}. A cholesterol internal standard was used to calculate RR_t and total amount of sterols. GC/MS analyses were performed with a CPSILSCB column, temp. programmed 250° to 320° at 5° min^{-1}. EIMS were obtained at 70 eV.

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