Phytochemistry, Vol. 27, No. 9, pp. 2765–2767, 1988.
Printed in Great Britain.

0031–9422/88 $3.00 + 0.00

TRANSFORMATION OF ISOVELLERAL BY THE PARASITIC FUNGUS *CALCARISPORIUM ARBUSCULA*

HEIDRUN ANKE and OLOV STERNER*

Department of Biotechnology, University of Kaiserslautern, D-6750 Kaiserslautern, F.R.G.; *Department of Organic Chemistry 2, Lund Institute of Technology, P.O.B. 124, S-221 00 Lund, Sweden

(*Received* 3 *February* 1988)

Key Word Index—*Calcarisporium arbuscula*, *Amblyosporium spongiosum*; parasitic fungi; Russulaceae; isovelleral; isovellerol.

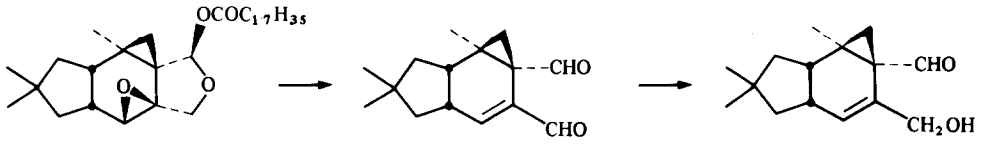
Abstract—The fungus *Calcarisporium arbuscula*, a parasite in fruit bodies of species of Russulaceae, resists high concentrations of the antifungal sesquiterpene isovelleral, a suggested defence compound formed enzymatically in many of its hosts as a response to injury. This insensitivity is suggested to be due to the ability of *C. arbuscula* to transform isovelleral, primarily to the reduced derivative isovellerol. Another fungicolous fungus, *Amblyosporium spongiosum*, which grows on the surface of the fruit bodies of many Basidiomycetes (including Russulaceae), also tolerates high concentrations of isovelleral, but does not transform the compound to the extent of more than a few per cent.

INTRODUCTION

Recent investigations of the enzymatic transformations of sesquiterpenes in injured fruit bodies of various species of Russulaceae (Basidiomycetes), have suggested that these may constitute a chemical defence system that preserves the fruit bodies from attack by parasites and microorganisms [1, 2]. The fruit bodies of *Lactarius vellereus*, for example, originally contain large amounts of stearoylvelutinal (**1**) which in seconds after an injury is transformed to unsaturated dialdehydes, e.g. isovelleral (**2**) [1]. While stearoylvelutinal (**1**) appears to have little if any biological activity [1], isovelleral (**2**), in common with many other unsaturated dialdehydes, has strong antimicrobial activity [1] and is also a potent direct-acting mutagen in the Ames test [3]. In addition, isovelleral (**2**) is a very pungent compound and has been shown to be an antifeedant against mammals that normally feed on mushrooms [4]. However, the unsaturated dialdehydes are not the end-products of the enzymatic conversions observed in the injured Russulaceae fruit bodies. Subsequently, one of the aldehyde groups is slowly reduced, whereby for instance isovelleral (**2**) is converted to isovellerol (**3**) [1]. This conversion is less conspicuous compared to the initial formation of the unsaturated dialdehydes, and its significance, if any, is not apparent. However, as the mutagenicity, the pungency, and the antimicrobial activities of, for example, **2** are diminished or disappear completely upon its reduction to **3** [1, 3], it is possible that the injured fruit bodies reduce the unsaturated dialdehydes in order to avoid prolonged contact with their own defence chemicals. The unsaturated dialdehydes are toxic in general, and may be harmful to the cells of the fruit body as well as to any parasite. When compounds **1** and **2** were screened for antifungal activity, **2** was found to be 10–100 times more potent against most fungi than **3** (C. Franzen and O. Sterner, unpublished results). However, the growth of the mycelium of *Calcarisporium arbuscula* Preuss was not inhibited even by high concentrations of isovelleral (**2**), and it is actually less sensitive to **2** than to isovellerol (**3**). *Calcarisporium arbuscula* is a parasite in fruit bodies, and is frequently found in species of Russulaceae in young and healthy specimens, where it grows endophytically without causing any visible symptoms [5]. However, when an infected fruit body gets older and starts to decay, the parasite emerges and produces its reproductive apparatus all over the surface of its host [5, 6]. Interestingly, the development of the fruit body and its production of spores is normally not affected by the presence of *C. arbuscula*, instead the parasite appears to make the fruit body less attractive to other parasites [5, 6].

RESULTS AND DISCUSSION

The concentrations of isovelleral (**2**) and isovellerol (**3**) causing 50% inhibition of the mycelial growth of *C. arbuscula* are given in Table 1. Strains originating from Europe, the U.S.A. and Australia show no differences in this respect. In order to investigate the fate of **2** in a



1 **2** **3**

Table 1. The concentrations (μg/ml) of isovelleral (2) and isovellerol (3) causing 50% inhibition of the mycelial growth on agar plates

	Isovelleral	Isovellerol
C. arbuscula 24–80 (F.R.G.)*	320	120
C. arbuscula 84–86 (U.S.A.)*	230	125
C. arbuscula 18–87 (Tasmania/Australia)*	240	140
A. spongiosum CBS 502.74.†	260	>1000

*Synthetic medium.
†YMG medium.

growing culture of the parasite, mycelium of *C. arbuscula* was grown in malt medium containing 0.2 mg **2** per ml. Each day a sample was extracted with ethyl acetate, and the presence of the compound was investigated by TLC. After three days at 22°, no trace of isovelleral could be detected, and comparison with a control culture (with no isovelleral) indicated that it had been replaced by essentially one compound. This was isolated and found to be **3**, identical in all respects with the isovellerol previously isolated from extracts of fruit bodies of *L. vellereus* [1]. As only 35% of the isovelleral (**2**) given to the cultures of *C. arbuscula* was recovered as isovellerol (**3**), other conversions of isovelleral, and/or additional metabolism of isovellerol (e.g. conjugation), yielding metabolites not extractable with ethyl acetate, probably take place. This could explain the fact that **3** is more toxic to the mycelium than **2** (see Table 1). The conidia of *C. arbuscula* do not appear to share the ability of the mycelium to transform isovelleral (**2**). The sensitivity of the conidia to **2** is of the expected order of magnitude, and considerably higher than the sensitivity to isovellerol (**3**) (see Table 2). In comparison with the mycelium, the conidia are formed in the final phase of the infection, and are obviously less likely to come in contact with isovelleral.

The sensitivity of another parasite on the Russulaceae fruit bodies, *Amblyosporium spongiosum* (Pers.) Hughes sensu Pirozynski, to **2** and **3** was also investigated. Unlike the mycelium of *C. arbuscula*, the mycelium of *A. spongiosum* does not grow endophytically, and it normally attacks the fruit bodies first after the onset of decay [7, 8]. As can be seen in Table 1, the relative sensitivity of the mycelium is reversed compared to *C. arbuscula*, although the fungus still tolerates high concentrations of isovelleral (**2**). When *A. spongiosum* was tested for its ability to transform isovelleral (**2**), only small amounts of isovellerol (**3**) (< 10% of theoretical yield) were detected after incubation for seven days. More than 90% of the isovelleral (**2**) was recovered unchanged from the culture, half of this amount was extracted from the mycelium and half from the culture broth. This shows that isovelleral (**2**) is taken up but not transformed to any great extent by the mycelium of *A. spongiosum*. The conidia of *A. spongiosum* show approximately the same sensitivity as those of *C. arbuscula* (Table 2).

Table 2. The minimal germination inhibiting concentration (μg/ml) of isovelleral (2) and isovellerol (3)

	Isovelleral	Isovellerol
C. arbuscula 24–80*	10	50
A. spongiosum CBS 502.74†	50	100

*10^5 conidia/ml synthetic medium.
†10^6 conidia/ml YMG medium [9].

In conclusion, *C. arbuscula* appears to have developed an ability to survive also in an environment containing high concentrations of the antifungal sesquiterpene isovelleral (**2**), by reducing it enzymatically to isovellerol (**3**). This should be advantageous for a parasite growing inside fruit bodies of species of Russulaceae. The parasite *A. spongiosum*, which grows on the surface of the fruit bodies, has not acquired this ability except in a minor way. Nevertheless, *A. spongiosum* tolerates high concentrations of **2**, although it has not been clarified how this parasite circumvents the toxicity of the compound.

In view of the transforming ability of *C. arbuscula*, the fact that a significant portion (*ca* 10%) of the Russulaceae fruit bodies have been reported to be infected by the parasite [5] must be considered when future investigations of the Russulaceae sesquiterpenes are made. However, the previously observed enzymatic conversions in injured Russulaceae fruit bodies, e.g. stearoylvelutinal (**1**) to isovelleral (**2**), and its subsequent reduction to isovellerol (**3**), are probably performed by the fruit bodies themselves. *Calcarisporium arbuscula* is not able to transform **1** to **2** and in addition, the same conversions have always been observed in injured fruit bodies, even if only one or a few specimens have been examined. It seems unlikely that infected specimens have been chosen every time, but for certainty this should be systematically investigated.

EXPERIMENTAL

The three strains of *C. arbuscula* were isolated from mushrooms collected in the F.R.G. in 1980 (strain 24–80), in the U.S.A. in 1986 (strain 84–86), and in Tasmania in 1987 (strain 18–87). The inhibition of their mycelial growth was assayed on agar plates (synthetic medium) with various concentrations of **2** and **3**. 5 mm discs of full-grown mycelium were placed in the centre of such plates. The radial growth of the mycelium compared with that on blanks was measured and the concns (μg/ml) causing 50% inhibition was estimated. The conidia were grown in synthetic medium with various concentrations of the two compounds, and the minimal germination inhibiting concns (μg/ml) were estimated. In order to determine the *C. arbuscula* transformation product of **2**, strain 24–80 was cultured in 200 ml malt medium (20 g/l) in 500 ml Erlenmeyer flasks at 22°. The medium contained 200 μg isovelleral per ml, and was inoculated with 20 ml of a malt medium pre-culture. After 3 days the cultures were extracted with EtOAc, which was dried with Na_2SO_4 and evapd. Chromatography of the extract on silica gel yielded pure isovellerol (**3**). *A. spongiosum* (CBS 502.74, isolated from an infected fruit body of *L. vellereus*), was grown in yeast-malt-glucose (YMG) medium [9]. The transformation experiment was carried out as described for *C. arbuscula*.

Acknowledgement—We thank A. Helfer for expert technical assistance. Support from the Alexander von Humboldt foundation (F.R.G.), and the Swedish Natural Science Research Council for O.S. is gratefully acknowledged.

REFERENCES

1. Sterner, O., Bergman, R., Kihlberg, J. and Wickberg, B. (1985) *J. Nat. Prod.* **48**, 279.

2. Sterner, O., Bergman, R., Franzen, C. and Wickberg, B. (1985) *Tetrahedron Letters* **26**, 3163.
3. Sterner, O., Nilsson, L. and Carter, R. (1987) *Mut. Research* **188**, 169.
4. Camazine, S., Resch, J., Eisner, T. and Meinwald, J. (1983) *J. Chem. Ecol.* **9**, 1439.
5. Watson, P. (1955) *Trans. Br. Mycol. Soc.* **38**, 409.
6. Nicot, J. (1968) *Bull. Trimest. Mycol. Soc. Fr.* **84**, 86.
7. Hawksworth, D. L. (1981) in *Biologi of Condial Fungi*, Cole, G. T. and Kendrick, B., eds.) Vol. 1, p. 171. Academic Press, New York.
8. Pirozynski, K. (1969) *Can. J. Botany* **47**, 325.
9. Rak, G., Anke, H. and Laatsch, H. (1982) *J. Antibiotics* **35**, 431.