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Phytochemistry 66 (2005) 817–823

PHYTOCHEMISTRY

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Laetiporic acids, a family of non-carotenoid polyene pigments from fruit-bodies and liquid cultures of *Laetiporus sulphureus* (Polyporales, Fungi)

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Received 2 December 2004; received in revised form 28 January 2005

Abstract

The non-isoprenoid polyene laetiporic acid A, recently described from fruit-bodies of the wood-rotting fungus *Laetiporus sulphureus*, was found to be the major orange pigment also in mycelium grown in liquid culture. Its formation was variable, ranging from 0.1 to 6.7 mg/g dry weight in three strains, all of which were identified as *L. sulphureus* by ITS rDNA sequence analysis. A second pigment, 2-dehydro-3-deoxylaetiporic acid A, is also described and fully characterized by NMR spectroscopy. Two further minor pigments, laetiporic acids B and C, were produced in liquid culture. These resemble laetiporic acid A but are enlarged by two and four carbon atoms, respectively, resulting in chromophores with 11 or 12 instead of 10 conjugated double bonds as described for laetiporic acid A. Since fruit-bodies of *L. sulphureus* are edible, laetiporic acids might hold potential as food colourants.

Keywords: *Laetiporus sulphureus*; Fruit-bodies; ITS sequences; Laetiporxanthin; Laetiporic acid; Polyene pigments

1. Introduction

The wood-rotting basidiomycete *Laetiporus sulphureus* (Bulliard:Fries) Murrill is among the most readily recognized of all macrofungi due to its striking yellowish or orange-coloured shelf-like fruit-bodies (Fig. 1(a)). The light absorption spectrum of the main pigment from fruit-bodies has a fine-structure typical of carotenoids (see Fig. 3), and the molecule was named laetiporxanthin and tentatively identified as 8′-apo-β-caroten-8′-oic acid by Valadon and Mummery (1969). Although no thorough structure elucidations were carried out, laetiporxanthin became generally accepted as the main *L. sulphureus* carotenoid pigment produced by fruit-bodies (Gill and Steglich, 1987) as well as in liquid culture (Soroka et al., 2002; Mishyn and Saroka, 2004).

Discrepancies between the proposed structure and our own spectroscopic data led us to re-investigate the orange pigment from *L. sulphureus* fruit-bodies using modern spectroscopic techniques (Weber et al., 2004). The pigment was found to be a polyene of non-isoprenoid biosynthetic origin, with the possible exception of the 2-methyl-3-oxo-1-butenyl terminal group, and was re-named laetiporic acid (**1**). Interestingly, the pigment featured a *cis* double bond at C-19 and occurred as a mixture of *cis–trans* isomers at C-7 in a 6:4 ratio. The conjugated polyene system responsible for light absorption in laetiporic acid contains 10 double bonds, a feature which is unprecedented among non-isoprenoid fungal pigments. In addition, the occurrence of a minor pigment, 2-dehydro-3-deoxylaetiporic acid (**2**), which would biosynthetically arise from laetiporic acid

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doi:10.1016/j.phytochem.2005.01.023

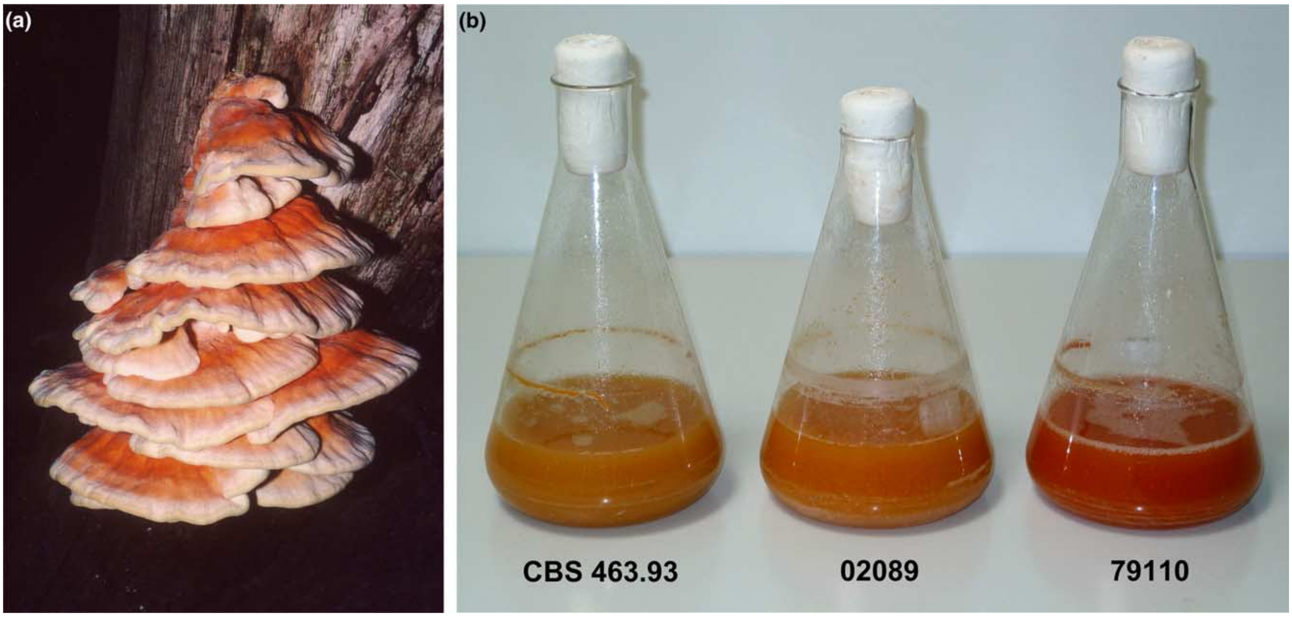

Fig. 1. *Laetiporus sulphureus*. (a) Fruit-bodies of strain 02089 growing on an old stump of *Quercus robur*, Sept. 2002. The same stump had already supported a flush of fruit-bodies in Sept. 1995. (b) Comparison of all three strains grown in liquid PCG medium in shaken flasks for 17 days.

through β-elimination of water, was suggested, but further investigations could not be performed due to the paucity of material (Weber et al., 2004).

Since liquid cultures of *L. sulphureus* also have a deep orange colouration, we were interested in establishing the identity of the pigment(s) synthesized under these conditions, quantifying them, and comparing these data with those from fruit-bodies. The analysis of liquid cultures also facilitated a comparison of different isolates of *L. sulphureus* under standard conditions, to establish the universality of pigment production in this species. These results are reported in the present paper. The structure elucidation of 2-dehydro-3-deoxylaetiporic acid (**2**) is also described. In addition, we report the discovery of two minor pigments, laetiporic acids B and C, with the original pigment **1** now named laetiporic acid A. Potential commercial applications of these substances as food colourants are briefly discussed.

2. Results and discussion

2.1. Pigment profile in liquid culture

Three strains of *L. sulphureus* were examined, viz. CBS463.93 (from fallen log of *Quercus rubra*; Baarn, Netherlands), 79110 (from dead wood of *Picea abies*; Tübingen, Southern Germany) and 02089 (stump of *Q. robur*; near Buxtehude, Northern Germany). The most intense pigment production was obtained in shaken-flask culture using the peptone–cornsteep–glucose (PCG) medium described by Mishyn and Saroka (2004) as compared to standard growth media. In marked contrast to the production of carotenoids by basidiomycete yeasts which is strongly enhanced by oxidative stress such as in well-aerated (indented) flasks as compared to standard conical flasks (Davoli et al., 2004), no such difference was observed with *L. sulphureus* (not shown).

Cultures of all three strains produced an identical pigment profile, with individual pigments identifiable by their HPLC–APCI-MS peaks. Laetiporic acid A (**1**) generated molecular ions at *m/z* 421 and 419 in the APCI-positive and -negative ionization modes, respectively, and was the major pigment. It eluted first under the chosen HPLC conditions (t_R 3.8 min) and showed an absorption peak at 445 nm in the UV/visible spectrum (Fig. 3). 2-Dehydro-3-deoxylaetiporic acid A (pigment **2**; *m/z* 403 and 401, respectively) was also present and eluted as a later peak (t_R 5.0 min; Fig. 3), as expected of a less polar molecule with an identical carbon skeleton. In addition, two minor pigments, laetiporic acids B (**3**) and C (**4**) were observed, eluting between **1** and **2** (t_R 4.1 and 4.4 min, respectively). The first of these (**3**) had a molecular mass of 446 (*m/z* 447 and 445 in the APCI-positive and -negative mass spectrum, respectively) and an absorption maximum at 456 nm whereas pigment **4** had a mass of 472 (*m/z* 473 and 471, respectively) and an absorption peak at 460 nm (Fig. 3). The increase in molecular weight by two increments of 26 mass units from **1** to **3** to **4**, accompanied by slightly higher retention times was suggestive of a stepwise extension of the polyene chain by two CH units, i.e., an additional double bond. The observed increase in λ_{max} from **1** to **3** to **4** indicated that these alkene units extended the chromophore of the molecule. Similar