Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 66 (2005) 817–823
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

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

Paolo Davoli [a], Adele Mucci [a], Luisa Schenetti [a], Roland W.S. Weber [b,*]

[a] *Dipartimento di Chimica, Università di Modena e Reggio Emilia, via Campi 183, I-41100 Modena, Italy*
[b] *Department of Biotechnology, University of Kaiserslautern, Paul-Ehrlich-Str. 23, D-67663 Kaiserslautern, Germany*

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

* Corresponding author. Tel.: +49 631 3167212; fax: +49 631 3167215.
E-mail address: rwsweber@rhrk.uni-kl.de (R.W.S. Weber).

0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.01.023

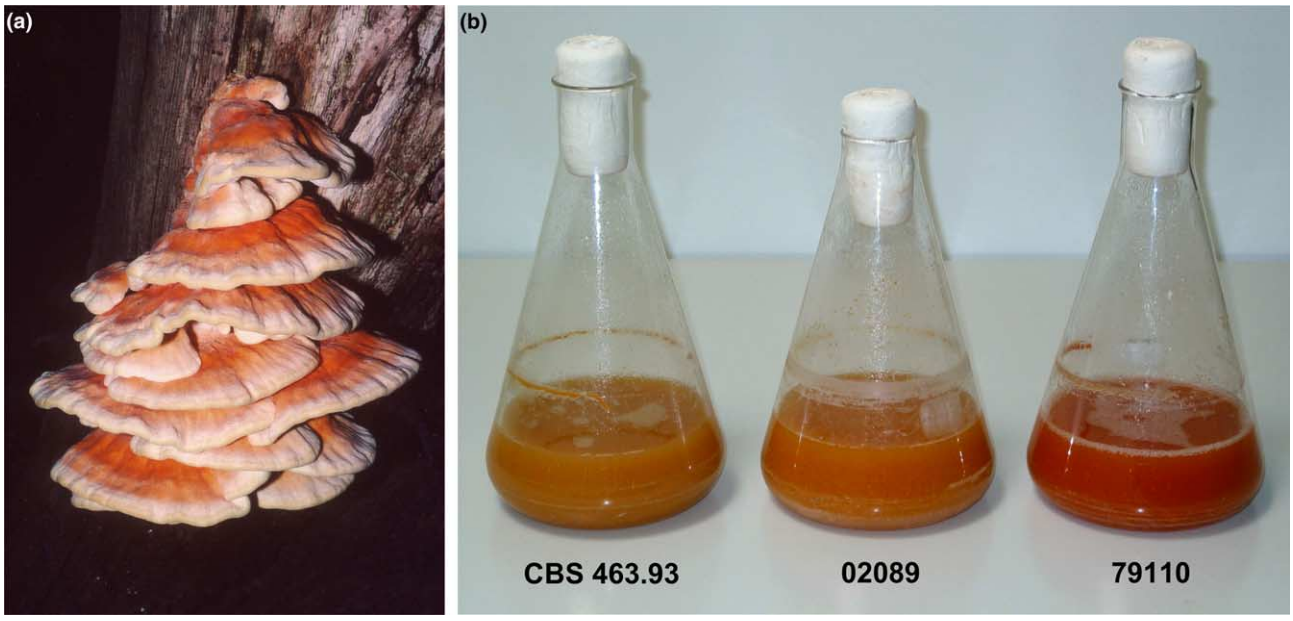

Fig. 1. *Laetiporus sulphureus*. (a) Fruit-bodies of strain 02089 growing on an old stump of *Quercus robur*, Sept. 2002. The same stump had already supported a flush of fruit-bodies in Sept. 1995. (b) Comparison of all three strains grown in liquid PCG medium in shaken flasks for 17 days.

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

bathochromic shifts due to extensions of the conjugated double-bond system are well known among carotenoids (Britton, 1995). Unfortunately, it was not possible to purify sufficient quantities of pigments **3** and **4** for complete structure elucidation by NMR. The elucidated structures of **1** and **2** are shown in Fig. 2.

2.2. Structure elucidation of pigments *1* and *2*

The major pigment produced in liquid culture had spectroscopic properties identical to those reported for laetiporic acid A (**1**) (Weber et al., 2004). A minor pigment with the same UV/visible spectrum and which differed of 18 mass units with respect to **1** had been detected also in *L. sulphureus* fruit-bodies, and the structure of 2-dehydro-3-deoxylaetiporic acid (**2**) was tentatively proposed on the basis of HPLC-MS data (Weber et al., 2004). The production of *L. sulphureus* pigments in liquid culture made possible the isolation of pigment **2** in a sufficient amount for NMR characterization which is reported below.

Similarly to laetiporic acid A (**1**), pigment **2** featured an overcrowded ^{1}H NMR spectrum in the olefinic region (5.7–7.2 ppm). Application of extensive homonuclear and heteronuclear techniques (gCOSY, gHSQC, gHMBC, ROESY, *J*-resolved) allowed us to assign unambiguously the structure of 2-dehydro-3-deoxylaetiporic acid A (24-methyl-25-oxo-hexacosa-2,5,7,9,11,13,15,17,19,21,23-undecaenoic acid, Fig. 2) to the minor *L. sulphureus* pigment **2**. When compared to **1**, no resonance around 4 ppm was observed in the ^{1}H NMR spectrum of pigment **2**, thus confirming the absence of the hydroxy group at C-3 which had been suspected from MS data. New signals at 5.87 and 7.07 ppm were detected as double triplets with $^{3}J = 15.7$ Hz, which well accounted for an additional double bond at C-2 with *trans* geometry. In addition, a triplet at 3.06 ppm correlating with four different olefinic carbon atoms in the gHMBC experiment (120.6, 129.5, 133.1 and 148.5 ppm) and showing a gHSQC correlation between a carbon signal at 35.1 ppm was suggestive of a CH_2 group which would keep the C-2–C-3 double bond isolated from the polyene chromophore, thus leaving the UV/visible spectrum of **2** unaffected with respect to laetiporic acid A, as experimentally observed (Fig. 3). The double triplet at 5.87 ppm (H-2) displaying an allylic coupling constant ($^{5}J = 1.8$ Hz) and that at 7.07 ppm showing a vicinal ^{3}J with the 4-CH_2 further corroborated the structure assignment of the acidic terminus of the molecule. As far as the opposite end group is concerned, all NMR data for the C-16–C-27 portion were in perfect agreement with those described for laetiporic acid A and confirmed the presence of a 1-methyl-2-oxo-1-propylidene terminal group in the *s-trans* conformation conjugated with the polyolefinic chain (Weber et al., 2004). The assigned ^{1}H and ^{13}C NMR data of 2-dehydro-3-deoxylaetiporic acid A (**2**) are reported in Table 1.

By analogy to laetiporic acid A, ROESY, *J*-resolved and gCOSY experiments showed that also the polyene chain in **2** contains double bonds with *cis* configuration. A *cis* double bond was clearly located between C-19 and C-20, i.e., at the same position as in **1** (Weber et al., 2004), as confirmed by the $^{3}J_{\text{H-19,H-20}} = 11.3$ Hz and by a diagnostic ROE between protons at 7.08

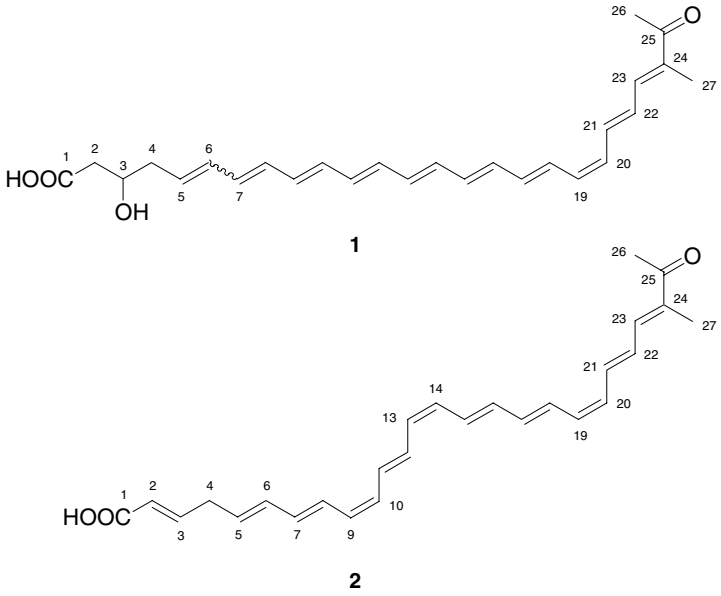

Fig. 2. Molecular structures of polyene pigments from liquid cultures of *Laetiporus sulphureus*. (**1**) Laetiporic acid A. This pigment was present predominantly (about 60%) as the *cis* isomer at C-7 (Weber et al., 2004). (**2**) 2-Dehydro-3-deoxylaetiporic acid A (major isomer; no information is available as to where the minor isomer configurationally differs).

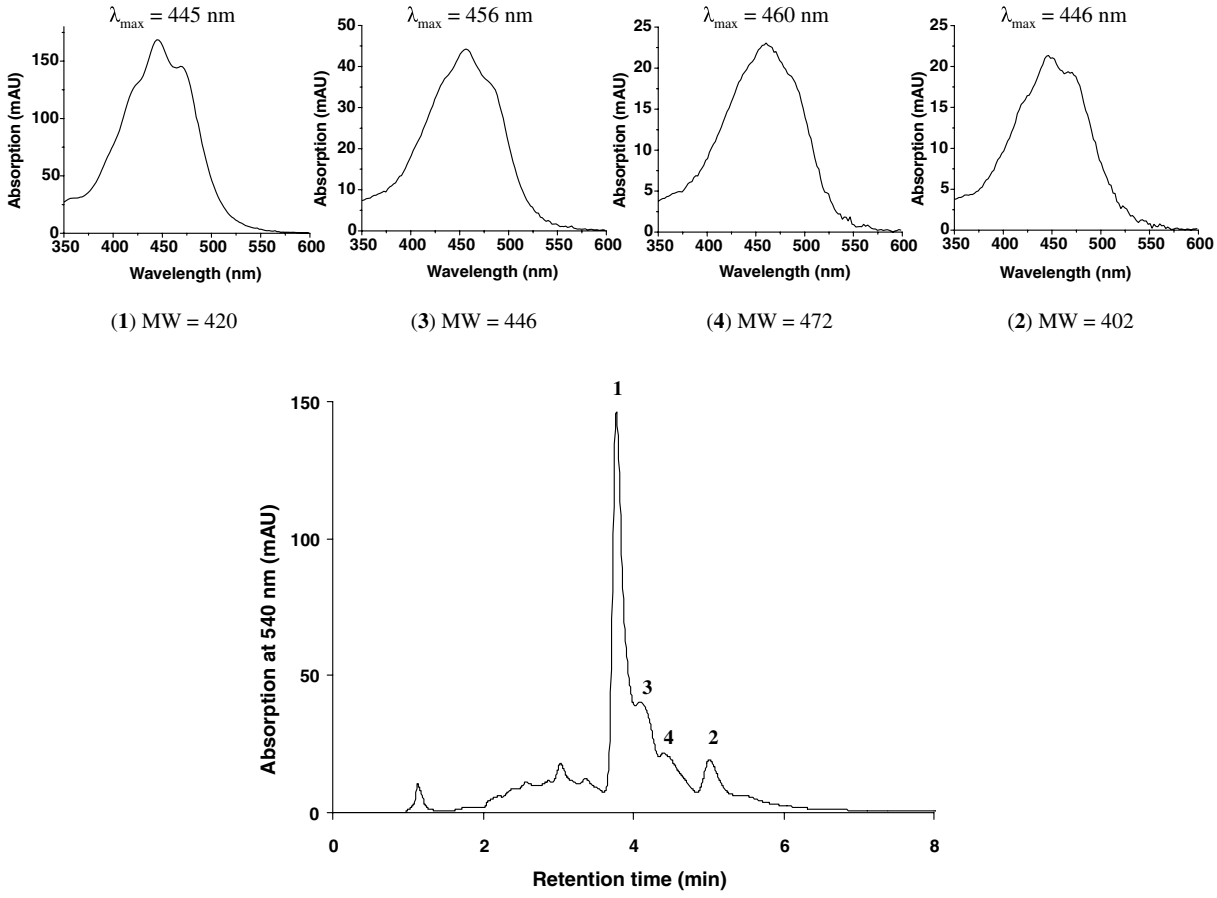

Fig. 3. UV/visible absorption spectra, molecular masses and HPLC chromatographic behaviour of pigments **1**–**4** in a crude mycelial extract of *L. sulphureus* strain 79110.

(H-21) and 6.81 ppm (H-18). Furthermore, NMR data suggested that two additional *cis* double bonds must occur at C-9 and C-13 in 2-dehydro-3-deoxylaetiporic acid A (**2**), even though clear-cut ROE effects could not be observed due to strong coupling and overlapping of proton signals. Nonetheless, the triplet at 6.07 ppm (H-9) with $^{3}J = 11.0$ Hz well supported a double bond at C-9 with *cis* geometry, whilst protons H-13 and H-14 showed highfield chemical shift values (6.12–6.15 ppm) that are closer to those observed for *cis* olefinic protons such as H-9,H-10 and H-19,H-20, thus allowing to locate a *cis* double bond also at C-13. In addition, it was possible to ascertain that the double bond between C-7 and C-8 bears *trans* stereochemistry in **2** ($^{3}J_{\text{H-7,H-8}}$ around 14.5 Hz), whereas in laetiporic acid A (**1**) it displayed opposite configuration in its major isomer (Weber et al., 2004). In this respect, a set of minor signals was detected in the ^{1}H NMR spectra of **2** (Table 1), which would suggest that also 2-dehydro-3-deoxylaetiporic acid A occurs as a mixture of geometric isomers, in ca. 8:2 ratio. Unfortunately, however, the small amount of sample available and, most importantly, the strong overlap of proton resonances in the olefinic region precluded any assignment as to the exact position where the two geometric isomers differ configurationally, and NMR signals for the minor isomer could be safely extracted for end groups only (Table 1).

Our initial explanation of the origin of the *cis* double bond at C-19 in pigment **1** from fruit-bodies was that of light-induced isomerization of a putative all-*trans* precursor, but this is not supported by the fact that the major isomers of both **1** and **2** in liquid culture not exposed to bright light were also in this configuration. The *cis* geometry at C-19 would therefore seem to arise at an early stage of biosynthesis, with the β-elimination of water to give **2** occurring as a later step by direct conversion of laetiporic acid A with 19-*cis* configuration (**1**). In contrast, whether the occurrence of double bonds with *cis* stereochemistry at positions other than C-19 may arise by isomerization of the corresponding *trans* analogues, either in vivo or as an artifact during isolation or storage, represents a topic for future investigations.

In the case of laetiporic acids B (**3**) and C (**4**), which could not be obtained in sufficient amount for any NMR structure elucidation, the question of their geometric configuration must remain unanswered at pres-

Table 1
^{1}H and ^{13}C NMR spectral data of 2-dehydro-3-deoxylaetiporic acid A (**2**) (major isomer)

Position	δ_H ($J_{H,H}$)	δ_C
1	–	n.d.
2	5.87 (1H, *dt*, 15.7, 1.8) [5.88 *minor*]	120.6
3	7.06_7 (1H, *dt*, 15.7, 6.6) [7.07_3 *minor*]	148.5
4	3.06 (2H, *t*, 6.6) [3.07 *minor*]	35.1
5	5.74 (1H, *dt*, 14.4, 6.7)	129.5
6	6.20 (1H, *dd*, 10.9, 14.4)	133.1
7	6.28 (1H, *dd*, 11.0, 14.4)	133.5
8	6.68 (1H, *dd*, 10.6, 14.6)	128.9
9	6.07 (1H, *t*, 11.0)	129.9
10	6.12 (1H, *m*[a])	130.1
11–12	6.76 (2H, *m*[a])	129.2
13–14	6.12–6.15 (2H, *m*[a])	130.2–131.1[b]
15	6.83 (1H, *dd*, 11.3, 14.7)	130.2
16	6.38 (1H, *dd*, 11.5, 14.1)	133.6
17	6.46 (1H, *dd*, 11.2, 14.2)	135.9
18	6.81 (1H, *dd*, 11.2, 14.8)	128.4
19	6.26 (1H, *t*, 11.3)	133.1
20	6.17 (1H, *t*, 11.4)	129.2
21	7.08 (1H, *dd*, 11.6, 14.8)	134.8
22	6.61 (1H, *dd*, 11.2, 14.9)	129.1
23	7.14 (1H, *d*, 11.4) [7.10 *minor*]	139.0
24	–	136.6
25	–	199.1
26	2.37 (3H, *s*)	25.5
27	1.93 (3H, *s*) [1.92 *minor*]	11.5

n.d., not detected.
Selected ^{1}H NMR resonances for the minor isomer are reported in square brackets.
[a] *J*-resolved spectroscopy indicates second order.
[b] Not resolved.

ent. By analogy to **1** and **2**, however, it might well be that laetiporic acids B and C feature a *cis* double bond at C-21 and C-23, respectively, i.e., at the same relative position to the ketonic terminal group. In this respect, future work on the biosynthesis of laetiporic acids should be of value to shed light on *cis–trans* isomerism in this family of fungal polyenes.

2.3. Quantification of pigment production

Purified pigments **1** and **2** were used to establish calibration curves under standard HPLC conditions. Although the mycelial biomass was similar between all three strains, the concentrations of both pigments were found to vary greatly (Table 2), reflecting the observed differences in colour intensity of the liquid cultures (Fig. 1(b)). Strain 02089 is of particular interest because this was isolated from the same fruit-body material (Fig. 1(a)) which was used for the original characterization of pigment **1** (Weber et al., 2004). The concentration of **1** was 3715 μg/g dry wt. biomass in liquid culture of strain 02089 as compared to 250 μg/g measured in its fruit-bodies (Weber et al., 2004). In comparison, the production of pigment **1** in mycelium grown in liquid culture was 6737 μg/g dry wt. in strain 79110, but only 107 μg/g in strain CBS463.93.

Table 2
Quantification of pigments **1** and **2** in liquid culture (400 ml PCG medium in 1-litre shaken conical flasks, 17 days cultivation at 24 °C)

Strain	Biomass (mg/ml)	Pigment **1** (μg/mg)	Pigment **2** (μg/mg)
CBS 463.93	2.8 ± 0.3	0.10 ± 0.02	Trace
79110	2.7 ± 0.2	6.7 ± 0.5	2.7 ± 0.4
02089	3.3 ± 0.1	3.7 ± 1.5	0.76 ± 0.39

2.4. Identification of the producing strains

All three strains formed fast-growing mycelium consisting of hyphae without clamp connections. Both thick-walled large chlamydospores and smaller thin-walled conidia were produced on agar and in liquid culture, with their appearance corresponding well with the description of *Sporotrichum versisporum* (Lloyd) Stalpers, the conidial state of *L. sulphureus*, as given by Stalpers (1984). No obvious morphological differences were observed between the three strains, except that the conidia of 79110 were unusually thick-walled. A comparison of the complete ITS1–5.8S rDNA–ITS2 sequences revealed 100% identity between strains CBS463.93 and 02089 (635 nt; deposited at GenBank under Accession No. AY835668), whereas strain 79110 (637 nt; GenBank AY835667) differed by 14 single-site substitutions or deletions, one 2-base deletion, and one 7-base insertion. GenBank searches yielded different *L. sulphureus* sequences as first hits for both sequences, at 99.7% identity (CBS463.93 and 02089) and 97.1% (79110). In the light of the unusually large ITS sequence diversity which has been observed between different isolates of *L. sulphureus* (Rogers et al., 1999; Banik and Burdsall, 2000), these results are interpreted as confirmation of species identity.

2.5. Potential commercial applications

The well-known antioxidant properties of carotenoid pigments are due chiefly to the conjugated double-bond system with its delocalized electron system. This allows carotenoids to absorb the excess energy of excited molecules such as singlet oxygen ($^{1}O_2$) followed by dissipation as heat, or to quench free radicals (Martin et al., 1999). Several studies especially with basidiomycetous red yeasts have indicated that carotenoid biosynthesis is stimulated by oxidative stress, including high aeration provided by indented flasks (Schroeder and Johnson, 1995; Sakaki et al., 2002; Bhosale, 2004; Davoli et al., 2004). By contrast, we did not observe any stimulation of laetiporic acid biosynthesis in *L. sulphureus* in indented flasks. Further, the purified pigments **1** and **2** were surprisingly stable in the presence of oxygen and light. Whereas carotenoids spotted onto paper or silica gel bleach within minutes or hours and decompose even in solvents such as acetone or toluene, pigments **1** and **2** were stable under these conditions for several months.

In this context, it may be worth mentioning that fruit-bodies of *L. sulphureus* have been used in the past for dyeing with yellow (Gillet, 1878; Cooke, 1898). Therefore, despite their polyolefinic nature, it is unlikely that pigments **1**–**4** have similar antioxidant activities as carotenoids.

However, their high stability might render laetiporic acids attractive as food dyes, not least because *L. sulphureus* is also known as an edible species with a long history of consumption especially in Continental Europe (e.g., Mattioli, 1554; Fries, 1874; Breitenbach and Kränzlin, 1986; Ryvarden and Gilbertson, 1993; Sanderson and Prendergast, 2002) and also in North America (e.g., Lincoff, 1981; Arora, 1986; Phillips, 1991). Metabolites produced by its fruit-bodies should therefore have GRAS (Generally Regarded As Safe) status.

3. Experimental

3.1. Organisms and culture conditions

Strain CBS463.93 was obtained from Centraalbureau voor Schimmelcultures (Utrecht, Netherlands). Strains 79110 and 02089 are maintained in the Culture Collection, Department of Biotechnology, University of Kaiserslautern. For pigment purification, fermentations were carried out in 2-litre conical flasks containing 1 litre PCG medium (3 g soy peptone, 3 g cornsteep, 15 g glucose, 0.6 g KH_2PO_4, 0.4 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$ per litre tap water; see Mishyn and Saroka, 2004) on an orbital shaker (120 rpm) at 24 °C for 17 days. For pigment quantification, 400 ml aliquots of PCG medium in 1-litre flasks were incubated for 28 days. YES medium (20 g sucrose, 4 g yeast extract, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$ per litre) and HMG medium (10 g glucose, 10 g malt extract, 4 g yeast extract per litre) were also tested but yielded inferior pigment production.

3.2. Extraction and purification of *1* and *2*

Mycelium of strain 79110 was separated from the culture fluid by Büchner filtration (depth filter T5500; Seitz-Schenk, Bad Kreuznach, Germany). For pigment quantification, freeze-dried weighed mycelium from 350 ml culture was extracted in 100 ml acetone. Samples were partially concentrated in vacuo to 25 or 50 ml and then analyzed by HPLC. For pigment purification, wet mycelium from 6.9 litre culture was extracted twice with 1 litre aliquots of acetone, followed by rotary evaporation to dryness and re-extraction by phase separation in water/ethyl acetate. The resulting crude extract was concentrated in vacuo and separated by silica gel chromatography (Kieselgel 60, 63–200 μm particle size; Merck, Darmstadt, Germany). Fractions enriched in **2** and **1** eluted in cyclohexane/ethyl acetate 1:1 and 1:3 (v/v), respectively. In order to separate the pigments from colourless rubbery impurities, both fractions were purified by passage over the size-exclusion resin Sephadex LH20 (Pharmacia, Uppsala, Sweden) in MeOH. In total, 6.08 mg pure pigment **1** and 1.92 mg **2** were obtained.

3.2.1. 2-Dehydro-3-deoxylaetiporic acid A (*2*)

Dark red amorphous solid. UV/visible λ_{max} (MeOH) nm (log ε): 421 (sh), 445 (4.59), 465 (sh). IR (KBr) ν_{max} cm^{-1}: 3432 s, 3019 m, 2924 m, 1652 s, 1388 m, 1281 m, 1227 m, 995 s, 602 w. For ^1H and ^{13}C NMR spectra, see Table 1.

3.3. HPLC chromatography and spectroscopic methods

A Hewlett-Packard (Waldbronn, Germany) HP 1090 Series II HPLC system equipped with a DAD detector and fitted with a LiChrospher 100 RP-18 column (5 μm particle size; 250 × 4 mm; Merck) was used for quantification of **1** and **2**. The injection volume was 10 μl and the flow rate 1 ml/min. The water/acetone gradient (Steel and Keller, 2000) was from 70% to 100% acetone in 15 min (Davoli et al., 2004). HPLC–APCI-MS analyses were performed in a similar water/acetone gradient using a Hewlett-Packard Series 1100LC-MSD instrument fitted with a LiChroCART Superspher 100 RP-18 column (4 μm particle size; 125 × 2 mm; Merck) in the APCI-positive and -negative ionization modes (see Davoli and Weber, 2002).

^1H and ^{13}C NMR spectra of purified **2** were recorded in $CDCl_3$ solution with a Bruker Avance 400 spectrometer at 400.16 and 100.61 MHz, respectively. Chemical shifts (δ) are reported in ppm downfield from TMS as reference; coupling constants ($J_{H,H}$) are given in Hz. Owing to the small amount of sample available in solution, ^{13}C resonances were not directly acquired, but measured in the inverse-detection mode with a spectral resolution of ±0.5 ppm. UV/visible spectra were measured in MeOH with a Lambda 16 spectrophotometer (Perkin–Elmer, Langen, Germany). Infrared (IR) spectra were recorded in KBr using an IFS-48 FT-IR spectrometer (Bruker, Karlsruhe).

3.4. Identification of fungi

For microscopic identification, the three strains of *L. sulphureus* were grown on cornmeal agar (Difco, Detroit, USA) and HMG agar (see above) at 24°C for 7–14 days. The cornmeal agar was poured thinly so as to permit the direct viewing of mounted squares with a Zeiss Axioskop 2 light microscope. For ITS sequence amplification, mycelium grown in HMG medium for 7 days was snap-frozen in liquid nitrogen and extracted using the phenol–chloroform protocol of Sacks et al. (1995). Amplification of the entire ITS1–5.8S rDNA–

ITS2 region of the ribosomal DNA cluster was carried out using the primers ITS5 (5′-GGAAGTAAAA-GTCGTAACAAGG) and ITS4 (5′-TCCTCCGCTTA-TTGATATGC) as described by Schwarz et al. (2004). GenBank searches were performed using the FASTA function of the GCG® Wisconsin® Package.

Acknowledgements

We are grateful to Prof. Timm Anke and Mrs. Anja Meffert (Department of Biotechnology, University of Kaiserslautern) for providing strain 79110 and for recording the mass spectra of pigments **1**–**4**, respectively. We thank Prof. Heidrun Anke (Institute of Biotechnology and Drug Research, Kaiserslautern) for valuable discussions, and Dr. Leanid Mishyn (Institute of Microbiology, Belarus National Academy of Sciences, Minsk) for kindly providing the medium composition for *L. sulphureus* used in his laboratory. We acknowledge support from the Fondazione Cassa di Risparmio di Modena for funding the purchase of the 400 MHz NMR spectrometer.

References

Arora, D., 1986. Mushrooms Demystified. Ten Speed Press, Berkeley, CA.

Banik, M.T., Burdsall, H.H., 2000. Incompatibility groups among North American populations of *Laetiporus sulphureus* sensu lato. Mycologia 92, 649–655.

Bhosale, P., 2004. Environmental and cultural stimulants in the production of carotenoids from microorganisms. Appl. Microbiol. Biotechnol. 63, 351–361.

Britton, G., 1995. UV/visible spectroscopy. In: Britton, G., Liaaen-Jensen, S., Pfander, H. (Eds.), Carotenoids, Spectroscopy, vol. 1B. Birkhäuser, Basel, pp. 13–62.

Breitenbach, J., Kränzlin, F., 1986. Fungi of Switzerland: Non-Gilled Fungi, vol. 2. Verlag Mykologia, Luzern.

Cooke, M.C., 1898. A Plain and Easy Account of British Fungi. W.H. Allen and Co., London.

Davoli, P., Weber, R.W.S., 2002. Identification and quantification of carotenoid pigments in aeciospores of the daisy rust fungus, *Puccinia distincta*. Phytochemistry 60, 309–313.

Davoli, P., Mierau, V.M., Weber, R.W.S., 2004. Carotenoids and fatty acids in red yeasts *Sporobolomyces roseus* and *Rhodotorula glutinis*. Appl. Biochem. Microbiol. 40, 460–465.

Fries, E.M., 1874. Hymenomycetes Europaei Sive Epicriseos Systematis Mycologici. Berling, Uppsala.

Gill, M., Steglich, W., 1987. Pigments of Fungi (Macromycetes). In: Herz, W., Grisebach, H., Kirby, G.W., Tamm, C. (Eds.), Progress in the Chemistry of Organic Natural Products, vol. 51. Springer, Wien, pp. 1–317.

Gillet, C.C., 1878. Les Champignons (Fungi, Hyménomycètes) qui Croissent en France – Description et Iconographie – Propriétés Utiles et Vénéneuses. Librairie J.-B. Baillière et Fils, Paris.

Lincoff, G.A., 1981. The Audubon Society Field Guide to North American Mushrooms. Alfred A. Knopf, Inc., New York.

Martin, H.D., Ruck, C., Schmidt, M., Sell, S., Beutner, S., Mayer, B., Walsh, R., 1999. Chemistry of carotenoid oxidation and free radical reactions. Pure Appl. Chem. 71, 2253–2262.

Mattioli, P.A., 1554. Commentarii in Libros Sex Pedacii Dioscoridis Anazarbei de Medica Materia. Valgrisio, Venezia.

Mishyn, L., Saroka, A., 2004. Effect of the nutrient medium composition on the production of carotenoids by the fungus *Laetiporus sulphureus* (Bull.:Fr.) Murr. in submerged culture. In: Present State and Development Prospects of Microbiology and Biotechnology, Proceedings of the International Conference, Minsk, 26–28 May, pp. 181–182.

Phillips, R., 1991. Mushrooms of North America. Little, Brown & Co., Boston.

Rogers, S.O., Holdenrieder, O., Sieber, T.N., 1999. Intraspecific comparisons of *Laetiporus sulphureus* isolates from broadleaf and coniferous trees in Europe. Mycol. Res. 103, 1245–1251.

Ryvarden, L., Gilbertson, R.L., 1993. European Polypores Lindtneria, vol. 1. Fungiflora, Oslo.

Sacks, W., Nürnberger, T., Hahlbrock, K., Scheel, D., 1995. Molecular characterization of nucleotide sequences encoding the extracellular glycoprotein elicitor from *Phytophthora megasperma*. Mol. Gen. Genet. 246, 45–55.

Sakaki, H., Nochide, H., Komemushi, S., Miki, W., 2002. Effect of active oxygen species on the productivity of torularhodin by *Rhodotorula glutinis* No. 21. J. Biosci. Bioeng. 93, 338–340.

Sanderson, H., Prendergast, H.D.V., 2002. Commercial uses of wild and traditionally managed plants in England and Scotland. The Royal Botanic Gardens, Kew, Richmond, Surrey, February 2002 Report, pp. 22–28.

Schroeder, W.A., Johnson, E.A., 1995. Singlet oxygen and peroxyl radicals regulate carotenoid biosynthesis in *Phaffia rhodozyma*. J. Biol. Chem. 270, 18374–18379.

Schwarz, M., Köpcke, B., Weber, R.W.S., Sterner, O., Anke, H., 2004. 3-Hydroxypropionic acid as a nematicidal principle in endophytic fungi. Phytochemistry 65, 2239–2245.

Soroka, O.N., Gvozdkova, T.S., Zalashko, M.V., 2002. Carotenoid synthesis by the fungus *Laetiporus sulphureus* M131 growing on different substrates. Vestsi Natsyyanal'nai Akademii Navuk Belarusi, Seryya Biyalagichnykh Navuk 4, 113–115 (in Russian); Chem. Abstr. 2003, 138, 365308.

Stalpers, J.A., 1984. A revision of the genus *Sporotrichum*. Stud. Mycol. 24, 1–105.

Steel, C.C., Keller, M., 2000. Influence of UV-B irradiation on the carotenoid content of *Vitis vinifera* tissues. Biochem. Soc. Trans. 28, 883–885.

Valadon, L.R.G., Mummery, R.S., 1969. A new carotenoid from *Laetiporus sulphureus*. Ann. Bot. 33, 879–882.

Weber, R.W.S., Mucci, A., Davoli, P., 2004. Laetiporic acid, a new polyene pigment from the wood-rotting basidiomycete *Laetiporus sulphureus* (Polyporales, Fungi). Tetrahedron Lett. 45, 1075–1078.