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NOTE

Albiducins A and B, salicylaldehyde antibiotics from the ash tree-associated saprotrophic fungus *Hymenoscyphus albidus*

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Since the early 1990s, European ash (*Fraxinus excelsior*) has been facing an onslaught of severe dieback in most of its natural distribution area in Europe. The causal agent of this epidemic disease is the invasive ascomycete *Hymenoscyphus fraxineus* (=*Hymenoscyphus pseudoalbidus*).[1–3] This fungal pathogen most probably originates from East Asia and has almost replaced the European sister species *Hymenoscyphus albidus*.[4,5] The latter species, *H. albidus*, is known in Europe since 1851[6] as a widespread, non-pathogenic decomposer of ash litter and produces numerous white-stalked apothecia on fallen, previous year's rachises of *F. excelsior*.[5,7] Apparently, *H. albidus* is threatened with local extinction in regions colonized by *H. fraxineus* due to competitive exclusion from its ecological niche (fallen petioles of *F. excelsior*).[4]

Over the past years, *H. fraxineus* has been studied intensively for virulence factors ultimately responsible for the pathogenicity. The role of previously described secondary metabolites, like the tetramic acid antibiotic hymenosetin,[8] during infection and necrosis formation has yet to be defined. Actually, the only known phytotoxins of *H. fraxineus,* viridiol and 3,4-dimethylpentan-4-olide, are, however, also produced by the non-pathogenic *H. albidus*.[9,10]

In the current study, we report the isolation and structure elucidation of two new polyketides, albiducins A and B (**1** and **2**, Figure 1), from liquid culture extracts of *H. albidus*. Further, these small salicylaldehyde derivatives were evaluated for cytotoxic and antimicrobial activities (Table 1).

The examined strain *H. albidus* 2009-111/1/4 was isolated from apothecia collected in 2009 in Bergen, Norway outside *H. fraxineus*-infected areas[11] and unambiguously determined by morphological characterization and sequencing of its ITS rDNA region (details see Supplementary Information). A 50 l scale fermentation of *H. albidus* 2009-111/1/4 was carried out for 20 days at 23 °C in ZM/2 medium. This medium was chosen because previous investigations had revealed its optimal composition for secondary metabolite production of *H. fraxineus*.[8] After cultivation had been stopped, the biomass was separated by centrifugation and extracted with acetone. The resulting mycelial extract was fractionated using normal-phase and reversed-phase column chromatography, yielding the two new bioactive compounds **1** and **2** (details see Supplementary Information).

Albiducin A (**1**) was isolated as yellow oil. Its molecular formula $C_{16}H_{20}O_3$ was determined by HRESIMS, indicative of seven units of unsaturation. The UV/Vis spectrum with absorption maxima of 226, 262, 298 and 398 nm indicated an extensive conjugated π system. The ^{1}H and ^{1}H,^{13}C HSQC spectra showed signals of an aldehyde, six aromatic/olefinic methines, four methylenes along one methyl group. In addition, the ^{13}C spectrum revealed the presence of four additional sp^2 hybridized quaternary carbons; two of which were bound to oxygen as indicated by their deep field chemical shifts. The nona-1,3-dienyl side chain was deduced from ^{1}H,^{1}H COSY and TOCSY correlations between protons H-8 to H_3-16 and confirmed by a multitude of intraresidue ^{1}H,^{13}C HMBC correlations (Supplementary Figure S1). HMBC correlations of H-8 to C-2/C-6, H-6 to C-2/C-4/C-8, H-5 to C-3/C-7 and H-1 to C-2/C-3/C-4/C-7 revealed the backbone of **1** (Supplementary Figure S1). Finishing the structure elucidation, the all-*trans*-geometry of the $\Delta^{8,9}$ and $\Delta^{10,11}$ double bonds was assigned based on the vicinal coupling constants ($J_{8,9}$ = 15.4 Hz; $J_{10,11}$ = 15.1 Hz). The IUPAC nomenclature of **1** is 2,3-dihydroxy-6-[(1*E*,3*E*)-nona-1,3-dien-1-yl] benzaldehyde.

Albiducin B (**2**) was analyzed having the molecular formula $C_{16}H_{20}O_2$, which indicated the formal loss of an oxygen atom compared to **1**. The proton and carbon spectra of **2** were very similar to those of **1**, with the key difference of the appearance of an additional aromatic methine instead of a quaternary carbon. This methine was part of the H-4/H-5/H-6 spin system. Again, $J_{H8,H9}$ = 15.6 Hz and $J_{H10,H11}$ = 14.9 Hz indicated all-*trans*-geometry of

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Dedicated to Professor KC Nicolaou and his outstanding contributions to complex natural product total synthesis and chemical biology.
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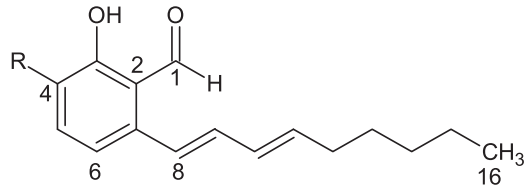

1: R = OH

2: R = H

Figure 1 Structures of albiducins A (**1**) and B (**2**).

Table 1 Antimicrobial and cytotoxic activities of albiducins A (1) and B (2)

Test organisms	*Albiducin A*	*Albiducin B*	*Reference*
Bacteria	MIC (μg ml^{-1})		
Bacillus subtilis DSM 10	16.7	66.7	4.2^{O}
Micrococcus luteus DSM 1790	33.3	66.7	0.4^{O}
Staphylococcus aureus DSM 346	33.3	66.7	0.1^{O}
Mycobacterium smegmatis ATCC 700084	n.i.	n.i.	2.1^{K}
Chromobacterium violaceum DSM 30191	n.i.	n.i.	0.4^{O}
Escherichia coli DSM 1116	n.i.	n.i.	1.7^{O}
Pseudomonas aeruginosa PA14	n.i.	n.i.	0.5^{G}
Fungi			
Candida albicans DSM 1665	66.7	n.i.	4.2^{N}
Pichia anomala DSM 6766	66.7	n.i.	4.2^{N}
Rhodotorula glutinis DSM 10134	16.7	n.i.	1.0^{N}
Schizosaccharomyces pombe DSM 70572	66.7	n.i.	16.7^{N}
Mucor hiemalis DSM 2656	33.3	n.i.	8.3^{N}
Cell lines	IC_{50} (μg ml^{-1})		
Mouse fibroblasts L929 (ACC 2)	6.1	25.0	0.0008^{E}
Human cervical carcinoma cell line KB3-1 (ACC 158)	2.7	8.5	—

Abbreviations: ^{E}, epothilone B; ^{G}, gentamicin; ^{K}, kanamycin; ^{N}, nystatin; n.i., no inhibition; ^{O}, oxytetracycline.

the $\Delta^{8,9}$ and $\Delta^{10,11}$ double bonds. The IUPAC nomenclature of **2** is 2-hydroxy-6-[(1*E*,3*E*)-nona-1,3-dien-1-yl]benzaldehyde.

Albiducins A (**1**) and B (**2**) are members of a group of structurally related small polyketides formally derived from salicylaldehyde (Supplementary Figure S2). Despite their relative simplicity, these small molecules have been found to possess a variety of interesting bioactivities. Their closest relative aurocitrin was isolated from *Hypocrea citrina* (currently valid name *Trichoderma citrinum*) and showed strong antibiotic activity against *Staphylococcus aureus*.[12] Furthermore, aurocitrin and its derivatives exhibited moderate antiplasmodial activities and cytotoxicity against three cancer cell lines.[13] Pyriculariol, produced by the rice blast fungus *Pyricularia oryzae*, was found to be phytotoxic due to dark necrotic spots caused on rice leaves and growth inhibition of rice seedlings.[14] 2,4-dihydroxy-6-((1*E*,3*E*)-penta-1,3-dien-1-yl)benzaldehyde from the endophytic fungus *Periconia atropurpurea* was able to induce slight increase in cell proliferation of HeLa and CHO cell lines and showed potent antifungal activity.[15] Another similar compound family, auroglaucin and its derivatives dihydroauroglaucin, isodihydroauroglaucin, flavoglaucin were isolated from '*Eurotium herbariorum*' (currently valid name *Aspergillus glaucus*) and displayed strong antioxidative activity.[16]

In our study, the antimicrobial activities of albiducins A (**1**) and B (**2**) were evaluated in a serial dilution assay against a broad panel of bacteria and fungi in a similar manner as previously reported[17] (Table 1, details see Supporting Information). Both compounds exhibited weak inhibitory activity against Gram-positive bacteria, including the pathogen *Staphylococcus aureus*. In addition, **1** displayed slight antifungal activity against all tested fungi. The cytotoxicity of **1** and **2** were assayed *in vitro* against the mouse fibroblast cell line L929 and the human cervical carcinoma cell line KB3-1. Both compounds showed moderate IC_{50} values with stronger effect on human cancer cells. In general, **1** was found to be more active than **2**. These results are in accordance with above described activities of previously reported structural related compounds.

Albiducin A (1): Yellow oil; UV (MeOH) λ_{max} (log ε): 226 nm (3.84), 260 nm (3.81), 296 nm (3.68), 398 (3.26); ^{1}H NMR (700 MHz, DMSO-d_6): δ_H 10.41 (s, H-1), 7.18 (d, J = 15.5 Hz, H-8), 7.03 (d, J = 8.4 Hz, H-5), 7.00 (d, J = 8.4 Hz, H-6), 6.66 (dd, J = 15.5 Hz, 10.4 Hz, H-9), 6.22 (dd, J = 15.1 Hz, 10.4 Hz, H-10), 5.86 (dt, J = 15.1 Hz, 7.1 Hz, H-11), 2.12 (q, J = 7.1 Hz, 7.1 Hz, H_2-12), 1.39 (dt, J = 14.0 Hz, 7.3 Hz, H_2-13), 1.33—1.25 (m, H_2-14, H_2-15), 0.87 (t, J = 7.0 Hz H_3-16); ^{13}C NMR (175 MHz, DMSO-d_6): δ_C 195.3 (CH, C-1), 150.6 (qC, C-3), 145.0 (qC, C-4), 135.7 (CH, C-11), 131.5 (CH, C-9), 131.3 (qC, C-7), 130.8 (CH, C-10), 125.6 (CH, C-8), 121.7 (CH, C-5), 118.2 (qC, C-2), 117.0 (CH, C-6), 32.2 (CH_2, C-12), 30.8 (CH_2, C-14), 28.4 (CH_2, C-13), 21.9 (CH_2, C-15), 13.9 (CH_3, C-16); ESIMS *m/z* 261.24 $[M+H]^+$, 259.10 $[M-H]^-$; HRESIMS *m/z* 261.1493 $[M+H]^+$ (calcd. for $C_{16}H_{21}O_3$, 261.1485); R_t = 14.7 min.

Albiducin B (2): Yellow oil; UV (MeOH) λ_{max} (log ε): 254 nm (3.58), 288 nm (3.34), 372 (2.94); ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 11.27 (br s, 3-OH), 10.46 (s, H-1), 7.45 (t, J = 8.0 Hz, H-5), 7.30 (d, J = 15.6 Hz, H-8), 7.14 (d, J = 8.0 Hz, H-6), 6.85 (m, H-4), 6.83 (m, H-9), 6.26 (dd, J = 15.1 Hz, 10.4 Hz, H-10), 5.95 (dt, J = 15.1 Hz, 7.2 Hz, H-11), 2.14 (m, H_2-12), 1.40 (m, H_2-13), 1.33—1.25 (m, H_2-14, H_2-15), 0.87 (t, J = 7.0 Hz, H_3-16); ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 194.4 (CH, C-1), 162.0 (qC, C-3), 140.8 (qC, C-7), 137.6 (CH, C-11), 136.1 (CH, C-5), 133.9 (CH, C-9), 130.7 (CH, C-10), 125.9 (CH, C-8), 118.1 (qC, C-2), 117.1 (CH, C-6), 115.6 (CH, C-4), 32.2 (CH_2, C-12), 30.9 (CH_2, C-14), 28.3 (CH_2, C-13), 21.9 (CH_2, C-15), 13.9 (CH_3, C-16); ESIMS *m/z* 245.20 $[M+H]^+$, 243.10 $[M-H]^-$; HRESIMS *m/z* 245.1535 $[M+H]^+$ (calcd. for $C_{16}H_{21}O_2$, 245.1536); R_t = 16.0 min.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)