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Piptamine, a New Antibiotic Produced by *Piptoporus betulinus* Lu 9-1

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In the course of our screening for antimicrobial metabolites of basidiomycetes we isolated piptamine **1** from a submerged culture of the mushroom *Piptoporus betulinus* Lu 9-1. Here we report the production, isolation, structure elucidation and biological activity of **1**.

The strain was isolated from a forested district near Neustadt/Orla (Thuringia, Germany) and deposited in the strain collection of the Hans-Knöll-Institute of Natural Products, Jena, Germany. A malt agar slant culture of *Piptoporus betulinus* Lu-9-1 was used to inoculate 500 ml Erlenmeyer flasks containing 100 ml of a seed medium composed of glucose 1%, malt extract 2%, soya bean meal 0.5%, yeast extract 0.1%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, $ZnSO_4 \cdot 7H_2O$ 0.0008%, $CaCO_3$ 0.1%, pH 5.0~5.3 (before sterilization). Cultivation occurred at 23°C and 110 rpm on a rotary shaker for 21 days.

Thereafter, 10 liters of the broth was extracted twice with 3 liter portions of ethyl acetate. The combined extract was dried and evaporated. The oily residue was subjected to column chromatography on Sephadex LH-20 (MeOH). Antibacterial fractions were detected by agar diffusion assay with *Bacillus subtilis* ATCC 6633 as test organism, pooled and evaporated to dryness. Final purification was achieved by column chromatography on silica gel 60 (0.063~0.1 mm) eluting with $CHCl_3$, $CHCl_3$/MeOH (9 : 1), $CHCl_3$/MeOH (8 : 2), and $CHCl_3$/MeOH (7 : 3). The bioactive fractions were evaporated to yield 35 mg of **1** as waxy solid. The Rf on TLC (silica gel aluminium sheets, $CHCl_3$/MeOH; 9 : 1 (v7v)) was 0.1.

The molecular weight of piptamine (**1**) and the chemical formula ($C_{23}H_{41}N$) were readily determined by HRESI-MS (Finnigan MAT 95 XL): *m/z* 332.3307 ($[M+H]^+$ 100%, calcd. 332.3298). Daughter ions were generated by CID-MS/MS of *m/z* 332.3307 (ESI-triple quadrupol instrument Quattro, VG Biotech, Altrincham, England). Thus diagnostic fragments with *m/z* 240.5 and *m/z* 90.7 were attributable to the methylpentadecylamino moiety and the benzyl group, respectively. In addition to *m/z* 332 ($[M+H]^+$) the ESI-MS of several products of other fermentations displayed *m/z* 304.3 ($[M+H]^+$, up to 30%) and *m/z* 360.4 ($[M+H]^+$, up to 15%), suggesting the occassional presence of chromatographically inseparable homologues. In accord with this contention CID-MS/MS of *m/z* 304.3 and *m/z* 360.4 furnished *m/z* 212.2 and *m/z* 90.7, respectively, and *m/z* 268.6 and *m/z* 90.6 as diagnostic fragments, suggesting that both minor components were distinguishable from **1** by a variable length of the side chain.

The structure of **1** was settled conclusively on the basis of one- and two-dimensional ^1H and ^{13}C NMR measurements (COSY, DEPT, HSQC, HMBC; Bruker Avance DRX 500, in $CDCl_3$, TMS as internal standard). The ^1H NMR spectrum of **1** displayed two aromatic protons at 7.50 ppm appearing as a doublet (H-3″, H-7″) and three other aromatic protons (7.55 ppm; H-4″, H-5″, H-6″) as a multiplet. Moreover, two methyl signals (H-15, H-1′) were visible at 0.95 ppm (triplet, 7.0 Hz) and 2.96 ppm (singlet). The tertiary amine structure of **1** was further confirmed by ^1H methylene signals at 4.52 ppm (H-1″) and 3.25 ppm (H-1, triplet, 7.4 Hz). The $^1H,^1H$-COSY coupling pattern suggested that H-1 and H-15 constituted the beginning and the end, respectively, of an aliphatic chain. In the ^{13}C NMR spectrum four aromatic carbon signals were visible. According to the ^1H and HSQC spectra three of these carbon signals (128.8, 132.9 and 130.2 ppm) are coupled to five protons (7.50 and 7.55 ppm) suggesting the occurrence of a singly substituted benzene ring. Most of the 15 carbon atoms of the aliphatic chain were discernible in the ^{13}C NMR spectrum with the exception of 28.9 ppm signal representing four carbons. Additional evidence for the structure of **1** was furnished by the C,H long-range coupled NMR spectrum (HMBC). Thus $^3J_{C,H}$ couplings of the *N*-methyl protons (H-1′) with C-1 and C-1″, and of H-1″ with C-2″, C-3″ and C-7″ were visible. The structure of **1** thus appears as a hitherto unknown fungal tertiary amine.

Fig. 1. Structure of piptamine (**1**).

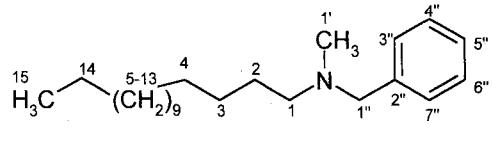

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Table 1. Antimicrobial activity of piptamine **1**.

Test organisms	MIC [μg/ml]
Staphylococcus aureus 134/94	6.25*
Staphylococcus aureus SG 511	0.78*
Enterococcus faecalis 1528	1.56*
Bacillus subtilis ATCC 6633	1.00*
Escherichia coli SG 458	12.5*
Rhodotorula rubra IMET 25030	50.0**
Kluyveromyces marxianus IMET 25148	6.25**
Candida albicans BMSY 212	6.25**
Sporobolomyces salmonicolor SBUG 549	6.25***
Penicillium notatum JP 36	> 50.0 ***

* MIC obtained by NCCLS-method[1)]

** MIC obtained by NCCLS-method[2)]

*** MIC obtained from agar well diffusion assay[3)]

Piptamine showed antimicrobial activity against a series of Gram-positive bacteria, yeasts and fungi (Table 1). Particularly notable MIC values for *Staphylococcus aureus* SG 511 and *Enterococcus faecalis* 1528, 0.78 μg/ml and 1.56 μg/ml, respectively, were obtained[1~3)]. Hemolytic activity of **1** was found at 10~50 μg/ml using heparinized blood of Beagle dogs[4)].

References

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