Anti-Staphylococcal Calopins from Fruiting Bodies of *Caloboletus radicans*

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ABSTRACT: Three new and seven known calopins were isolated from *Caloboletus radicans*. The structures of the new cyclocalopins, 8-deacetylcyclocalopin B (**1**), cyclocalopin A-15-ol (**2**), and 12,15-dimethoxycyclocalopin A (**3**), were mainly elucidated by NMR and MS data analysis. The stereochemistry of **1**–**3** was assigned based on ROE correlations, coupling constants and by comparison of their CD spectra with those of similar known calopins. While **1**–**10** were inactive against two cancer cell lines, they displayed antistaphylococcal activity against methicillin-resistant *Staphylococcus aureus* strains (MRSA) with MIC values of 16−256 μg/mL. Moreover, some calopins were active against the fish pathogen *Enterococcus faecalis* F1B1.

Antibiotic resistance is one of the major challenges of today's global public health. Bacteria resistant to antibiotics are described as "nightmare bacteria" by the World health leaders that "pose a catastrophic threat" to people in every country in the World.[1] Among the antibiotic resistant pathogens, methicillin-resistant strains of *Staphylococcus aureus* (MRSA) are spreading worldwide within hospitals, extended care facilities and the community at large. Examples of infections caused by MRSA include respiratory, skin, soft tissue, bone, joints and urinary tract infections, endocarditis, acute osteomyelitis, meningitis, neonatal liver abscesses, epidural abscesses, mastitis, toxic shock syndrome, and even chorioamnionitis.[2] According to a recent report, more than 25% of *S. aureus* infections in Europe are caused by MRSA.[3] MRSA is contagious and can be spread through direct or indirect contact with an infected person. The treatment of these infections is expensive, problematic and time consuming. Moreover, long-term antibiotic medication to treat bacterial infections causes numerous side effects.[4] These facts indicate the urgency to discover sufficiently active new natural scaffolds for the development of antibiotics with less or no side effects. It is well known that nature produces an amazing variety and number of compounds, which are both a fundamental source of new chemical diversity and an integral component of today's pharmaceutical compendium.[5-7] However, readily available sources of natural products are often well investigated. Therefore, our search for new bioactive natural products is focused on mushrooms which are not easily accessible, since they usually cannot be cultivated and only occur temporarily in their natural environment.[8,9] This also applies to *Caloboletus radicans* (Pers.) Vizzini, (= *Boletus radicans* Pers.: Fr., German name: Wurzelnder Bitterröhrling). The German name of this species reveals that the fruiting bodies taste bitter and are consequently inedible. Several years ago the bitter taste of the fruiting bodies was attributed to the presence of the previously unknown calopins.[10-12] Although even total syntheses of several calopins have been published,[13-15] there are no reports on the bioactivity

of the calopins. The free radical-scavenging activity against DPPH,[16,17] superoxides,[17] and PABS[17] radicals has been reported.

In this manuscript, we describe the isolation and structure elucidation of new (**1**–**3**) and previously reported calopins (**4**–**10**) from fruiting bodies of *C. radicans* which turned out to exhibit anti-staphylococcal activity and activity against fish-pathogenic bacteria.

Fractionation of the MeOH–H_2O extract from freeze-dried defatted fruiting bodies of *C. radicans* by first partitioning with n-hexane and EtOAc, followed by repeated reversed-phase HPLC of the EtOAc soluble material with a subsequent MeCN–H_2O gradient and MeOH–H_2O isocratic eluents yielded three new cyclocalopin derivatives **1**–**3** and seven previously known calopins **4**–**10**.

The known calopins were identified as cyclopinol (**4**), cyclocalopin B (**5**), cyclocalopin A (**6**), 15-methoxycyclocalopin A (**7**), cyclocalopin E (**8**), calopin (**9**) and *O*-acetylcalopin (**10**) by comparison of their ^{1}H NMR, ^{13}C NMR, ESIMS and CD data to those reported previously.[10-12]

The molecular formula $C_{17}H_{24}O_7$ of **1** was deduced by high-resolution ESIMS of the pseudomolecular ion. Seventeen carbon resonances were observed in the ^{13}C NMR spectrum which were assigned with the help of the HSQC spectrum to one ester carbonyl group at δ_C 173.9 ppm, one acetyl carbon at δ_C 176.0 ppm, two sp^2 carbons at δ_C 133.3 and 123.1 ppm, two oxygenated methane carbons at δ_C 71.9 and 77.5 ppm, one oxygenated carbon with acetyl group at δ_C 73.5 ppm, one oxygenated methylene carbon at δ_C 72.9 ppm, one oxygenated quaternary carbon at δ_C 108.3 ppm, one methylene carbon at δ_C 27.2 ppm, two methine carbons at δ_C 30.3 and 47.1 ppm, one quaternary carbon at δ_C 52.9 ppm and four methyl carbons at δ_C 14.6, 17.5, 19.5 and 21.1 ppm. Based on these observations, the planar structure of **1** was established with the help of COSY, HMBC and ROESY correlations (Figure 1). A comparison of the NMR data of **1** with those of the known compound **5**

revealed that **1** is structurally closely related to **5**.[10] The new calopin (**1**) differs from compound **5** by the absence of one of the two acetyl groups of **5**. The acetyl group of **1** was shown to be attached to C-7 by an HMBC correlation of H-7 at δ_H 5.07 ppm to the acetyl carbon at δ_C 173.9 ppm (Figure 1). The coupling constants in the ^{1}H NMR and the CD data of **5**[10] are similar to those of **1.** Furthermore, the NOE correlations (Figure 1) of H-3 with H-8, of H-7 with H-13, and of H-11α with H-14 in the NOESY spectrum of **1** indicate that 8-deacetylcyclocalopin B (**1**) has the same configuration as cyclocalopin B (**5**).

The molecular formula of **2** was determined to be $C_{15}H_{20}O_7$ by high-resolution ESIMS of the pseudomolecular ion. The ^{1}H and ^{13}C NMR data (Table 1) in combination with the molecular formula indicated that **2** is structurally closely related to cyclocalopin A (**6**).[10] Instead of the methyl group present in **6**, **2** bears a CH_2OH group at C-9. The position of the CH_2OH group at C-9 was deduced from the HMBC correlations of H-10 at δ_H 6.96 ppm with the methylene carbon C-15 at δ_C 60.2 ppm, and of H-15 at δ_H 4.30 ppm with the carbonyl carbon C-8 at δ_C 199.3 ppm (Figure 1). Due to the observed NOE correlations (Table 1), nearly identical coupling constants, and CD spectra cyclocalopin A-15-ol (**2**) possesses the same configuration as **6**.

The molecular formula $C_{17}H_{24}O_7$ of **3** was derived from the pseudomolecular ion in the high-resolution ESIMS in combination with ^{1}H and ^{13}C NMR data. The ^{1}H and ^{13}C NMR data (Table 1) of **3** are very similar to those of 15-methoxycyclocalopin A (**7**).[12] Compound **3** differs from **7** only in respect of the presence of two broad singlets at δ_H 3.17 and 3.36 ppm instead of one, revealing that **3** possesses two methoxy groups. One CH_3O- group is attached at C-12 due to HMBC correlations of H-17 at δ_H 3.17 ppm with the anomeric carbon C-12 at δ_C 110.9 ppm and with the carbon C-6 at δ_C 56.7 ppm (Figure 1). The second CH_3O- group is located at C-15 as shown by the HMBC correlation of H-16 at δ_H 3.36 ppm with the methylene carbon C-15 at δ_C 68.6 ppm. The stereochemistry of 12,15-dimethoxycyclocalopin

A (**3**) was assigned to be the same as that of **7** because of nearly identical coupling constants and CD data.

Calopins **1**–**10** (100 μM) were inactive against PC3, prostate cancer and HepG2, liver hepatoblastoma cells. The activity of **1**–**10** against a panel of *S. aureus* strains, including the multidrug resistant (MDR) and methicillin-resistant *Staphylococcus aureus* (MRSA) strain SA-1199B that overproduces the NorA efflux pump and possesses a high level of resistance to some fluoroquinolones,[18,19] the macrolide-resistant strain RN4220,[20] the MRSA strain XU212 that is resistant to tetracycline,[21] the standard laboratory strain ATCC 25923,[21] and the epidemic methicillin-resistant strain EMRSA-15 was evaluated.[19] Calopins **1**–**10** displayed activity against SA-1199B but turned out to be inactive against the strain RN4220 (Table 2). Moreover, **1** was found to be more active than the positive control norfloxacin.[19-22] against ATCC 25923 and SA-1199B with MICs of 16 μg/mL. In general, compound **3** was found less active than the calopins **1** and **2**. The reason might be the lack of a free hydroxy group at C-12.

Compounds **3, 5, 6, 9** and **10** were also tested against the phytopathogens *Phytophthora capsici*, a pathogen of several plants including peppers,[23] *Colletotrichum gloeosporioides* strain CG12, a phytopathogen of strawberry,[24] *Magnaporthe oryzae Triticum* pathotype strain JP4,[25] a wheat blast fungus, and against the two fish pathogenic *Enterococcus faecalis* strains[26] FF11 and F1B1. The tested compounds displayed varying levels of inhibitory effects against the strain F1B1 at 40 μg/disk but were inactive against *C. gloeosporioides*, *M. oryzae Triticum* pathotype and *E. faecalis* strain FF11 up to 1000 μg/ml. The inhibition zones around the disks were recorded to be 5.5 ± 0.9 for compound **3**, 5.5 ± 1.0 for **5**, 7.1 ± 1.5 for **6**, 5.6 ± 1.3 for **9**, and 9.5 ± 1.2 mm for compound **10**. Since the fish pathogenic *E. faecalis* strain F1B1 is highly resistant to several antibiotics such as amoxicillin, ampicillin, cefradine, cefuroxime, erythromycin and penicillin G,[26] the calopins might be useful as lead structures

for the development of agents for the control of fish pathogens.

EXPERIMENTAL SECTION

General Experimental Procedures. Extraction was performed in a 5 L Erlenmeyer flask with constant stirring using an IKA magnetic stirrer. Filtration was carried out with filter paper with a particle retention size of 12–15 μm. Organic solvents were evaporated under reduced pressure using a rotary evaporator (IKA) at room temperature (26 °C). A benchtop manifold freeze-drier from Christ was used to obtain completely H_2O free samples. Semi-preparative HPLC separation was performed on an HPLC system equipped with two Waters 590EF pumps, the automated gradient controller 680 and a Knauer UV/vis detector. Extracts from *C. calopus* were separated first on a C_{18} ec column (Nucleodur, 16 × 250 mm, 5 μm, 100 Å, Macherey-Nagel) with the following gradient: Starting from MeCN–H_2O (+0.1% HOAc) (10:90) linear to MeCN–H_2O (+0.1% HOAc) (40:60) within 30 min, then within 20 min to 100% MeCN; flow rate 6 mL/min; UV detection at 250 nm. A second separation step was performed on a C_{18} ec column with a smaller diameter (Nucleodur, 10 × 250 mm, 5 μm, 100 Å, Macherey-Nagel) with the following isocratic separation program: MeOH–H_2O (20:80); flow rate 3 mL/min; UV detection at 250 nm. Optical rotations: Perkin-Elmer 243. CD: Applied Photophysics Chirascan^{TM} CD spectrometer (1 mm cell). NMR: Bruker Avance DRX-600 (^{1}H NMR at 600.22 MHz, ^{13}C NMR at 150.91 MHz, 300 K), and Bruker Avance WB-360 (^{1}H NMR at 360 MHz, ^{13}C NMR at 91 MHz, 300 K); chemical shifts in ppm are referenced to the residual solvent signal (D_2O: δ_H = 4.79 ppm; $CDCl_3$: δ_H = 7.26 ppm, δ_C = 77.0 ppm). LC-ESIMS spectra were obtained on an LCQ DecaXP Plus ESIMS spectrometer (Thermo Fisher Scientific Inc., USA). The spectrometer was operated in the positive mode (0.625 spectra s^{-1}; mass range 50−1000). Nitrogen was used as sheath gas (80 arbitrary units) and helium served as collision gas. The spectrometer was equipped with a Hewlett-Packard

HPLC system (Series 1100) consisting of a degasser, a pump system, a DAD detector, and an auto-sampler (injection volume 10 μL). The separations were performed with a C_{18} ec column (Nucleodur, 4.6 × 250 mm, 5 μm, 100 Å, Macherey-Nagel) using the following gradient program: Within the first 50 min linear from MeCN–H_2O (+0.1% HOAc) (10:90) to 100% MeOH, flow rate 0.66 mL/min. HR-ESIMS spectra of compounds **1** and **2** were recorded on a Finnigan MAT 95 high resolution, double focusing magnetic sector field mass spectrometer. Accurate mass measurements were achieved using the accelerating voltage of 5 kV, taurocholic acid as an internal standard, ($[M–H]^-$ = 514.2839), a resolution between 5000 and 10000, and a scan range of 150 to 1000 *m/z*. The HR-ESIMS spectrum of compound **3** was acquired with a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific, Bremen, Germany), using a heated electrospray ionization (HESI-II) source in positive mode at a flow-rate of 5 μL per minute. MS measurement was performed in full MS mode with a resolution of 280,000, a scan range of 150 to 800 *m/z* using a spray voltage of 3 kV.

Collection of Mushrooms. Fruiting bodies of *C. radicans* Pers.: Fr. (leg. et det. N. Arnold) were collected in September and October 1998 at Gerolfing in Bavaria, Germany and immediately frozen and stored at −20 °C. Voucher samples of *C. radicans* are deposited at the Institut für Organische und Analytische Chemie, Universität Bremen, Germany.

Extraction and Isolation. Frozen fruiting bodies of *C. radicans* (2.0 kg) were crushed and extracted with 2 L of MeOH−H_2O (1:1 v/v) (two times) with constant stirring at 200 rpm and 25 °C for 1 h. The supernatant was filtered, solvent was evaporated, residue re-dissolved in MeOH−H_2O (1:4 v/v) and partitioned between n-hexane and EtOAc. The solvents from n-hexane (Hx), EtOAc and MeOH−H_2O (MH) fractions were evaporated and subjected to an LC-MS and ^{1}H NMR analysis. The LC-MS and ^{1}H NMR data of the Hx and MH fractions clearly indicated the presence of fat and sugar, respectively, whereas the EtOAc fraction contained several classes of compounds including nucleosides. The EtOAc fraction (455.6

mg) was subjected to further purification. The EtOAc fraction was dissolved in methanol and filtered with a C_{18} ec cartridge. The supernatant was separated by HPLC first on an C_{18} ec column with 16 mm diameter, on an C_{18} ec column with 10 mm diameter. A 2 kg amount of fruiting bodies yielded 2.8 mg of 8-deacetylcyclocalopin B (**1**), 2.3 mg of cyclocalopin A-15-ol (**2**), 5.3 mg 12,15-dimethoxycyclocalopin A (**3**), 2.5 mg of **4**, 8.2 mg of **5**, 12.5 mg of **6**, 3.5 mg of **7**, 1.8 mg of **8**, 15.5 mg of **9** and 22.8 mg of **10**.

8-Deacetylcyclocalopin B ***(1)****:* colorless oil; $HPLC_{prep}$, t_R 23.8 min (step 1), 30.5 min (step 2); LC-(+)-ESIMS t_R 24.5 min; $[\alpha]^{24}_D$ −0.21 (*c* 0.003, MeOH); UV/Vis (MeOH) λ_{max} 256, 268, 274 nm; CD (CH_3CN) λ_{max} ($\Delta\varepsilon$) 221 (+0.77), 231 (+0.38), 239 (0), 251 (−0.19), 271 (−0.05) nm; ^1H and ^{13}C NMR (see Table 1); HR-(+)-ESIMS *m/z* 363.1409 $[M + Na]^+$ (calcd for $C_{17}H_{24}O_7Na^+$, 363.1419 $[M + Na]^+$).

*Cyclocalopin A-15-ol (***2***):* amorphous solid, $HPLC_{prep}$, t_R 30.6 min (step 1), 35.5 min (step 2); LC-(+)-ESIMS t_R 30.9 min; $[\alpha]^{24}_D$ −46.5 (*c* 0.05, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 214 (+3.69), 223 (+1.55), 228 (0), 243 (−3.20), 253 (−2.01), 323 (+0.87) nm; ^1H and ^{13}C NMR (see Table 1); HR-(−)-ESIMS *m/z* 311.1135 $[M - H]^-$ (calcd for $C_{15}H_{19}O_7^-$, 311.1131 $[M - H]^-$).

12,15-Dimethoxycyclocalopin A ***(3)****:* amorphous solid, $HPLC_{prep}$ t_R 20.52 min (step 1), 22.30 min (step 2); LC-(+)-ESIMS t_R 22.85 min; $[\alpha]^{24}_D$ +35.5 (*c* 0.01, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 216 (+4.95), 220 (+0.55), 228 (0), 243 (−4.20), 323 (+0.87) nm; ^1H and ^{13}C NMR (see Table 1); HR-(+)-ESIMS *m/z* 363.1409 $[M + Na]^+$ (calcd for $C_{17}H_{24}O_7Na^+$, 363.1419 $[M + Na]^+$).

Test for Anti-Staphylococcal Activity. The anti-MRSA activity of the compounds **1**−**10** was investigated with the standard MRSA strains ATCC 25923, XU212, SA-1199B, RN4220, and EMRSA-15 by a broth dilution assay.[27] All five MRSA strains were incubated separately for 24 h at 37 °C on nutrient agar (Oxoid) and prepared for an inoculum density of 5×10^5 colony forming units in normal saline water (9 g/L) by comparison with a 0.5 MacFarland turbidity

standard. To avoid contamination, sterilized nutrient agar, saline water and Mueller-Hinton broth (MHB; Oxide) were used. The positive control (norfloxacin) was prepared by dissolving the antibiotic in DMSO (Sigma). After having all these requisites in hand, MHB was dispensed into 10 wells of a 96 well microtiter plate (Nunc, 0.3 ml volume per well). The compounds **1**−**10** and antibiotic were serially diluted (over the range of concentrations 0.5−256 μg/mL) into each of the wells followed by the addition of the bacterial inoculum, and the microtiter plate was incubated at 37 °C for 18 h. A DMSO control was included in all assays. The MIC recorded the lowest concentration at which no growth was observed. This was facilitated by the addition of 20 μL of a 5 mg/mL methanolic solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) to each of the wells and incubation for 20 minutes. Bacterial growth was indicated by a color change from yellow to dark blue. All MICs were determined independently at least three times.

Activity against Oomycetes and Fungi by a Disk Diffusion Method.[28] To test the inhibitory activity of the compounds **3, 5, 6, 9** and **10**, the oomycete *Phytophthora capsici* and the fungi *Colletotrichum gloeosporioides* and *Magnaporthe oryzae triticum* Pathotype were cultured on 10% V8 agar medium at 25 °C in the dark.[29] A 4 mm diameter mycelia agar block was placed at the center of the PDA plate (9 cm). The compounds were dissolved in acetone and a stock solution of 1000 μg/mL was prepared. A 40 μL sample of each stock solution was carefully applied onto a paper disk (8 mm) and allowed to dry. After evaporation of the solvent, the disk was placed 4 mm apart from the mycelia block of the pathogen and incubated at 25 °C in the dark for 5 days. Then, the zone of inhibition was measured (mm) for each sample against control strains. Each treatment was replicated three times.

Inhibitory Activity against Fish Pathogens by a Disk Diffusion Method.[28] To test the antibacterial activity of compounds **3, 5, 6, 9** and **10** against *Enterococcus faecalis* strains FF11 and F1B1, bacterial suspensions (1×10^5 CFU/mL) were spread on the agar plate. A 40

µL sample of each compound (concentration 1000 µg/mL) in acetone was carefully applied onto a paper disk (8 mm) and allowed to dry the solvent and then placed onto the MH agar plate. The inhibitory activity of the compound is displayed as bacteria free halo zone after 2 days of incubation at 37 °C. The diameter of the halo zone was measured and the result was expressed as mean value ± standard error of three replications of each dose of each compound. Antibiotic azithromycin was used as positive control.

Cytotoxicity Test (SRB Assay). The cancer cell growth inhibitory activity of the compounds **1**−**10** was evaluated by a sulforhodamine B (SRB) assay.[30,31] In brief, prostate cancer (PC3) and liver hepatoblastoma (HepG2) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) media supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (USA) and maintained at 37 °C in a humidified incubator with an atmosphere of 5% CO_2. The cells were seeded in 96-well microtiter plates in 100 µl culture medium at cell concentration of 5×10^4 cells/mL. After an overnight adaptation period, the cells were treated with test compounds **1**−**10** in a CO_2 incubator for 72 h. When cells reached 70% confluence in a microtiter plate, the medium was replaced with DMEM 5% FBS and incubated further 48 h. Then, the medium was discarded, and cells were fixed by adding 50 μL of 10% trichloroacetic acid (TCA, Sigma-Aldrich Chemical, St. Louis, MO, USA). The cells were then incubated at 4 °C for 30 min; TCA was drained off, and the plates were left to dry. Then, 50 μL of SRB stain (10 mg of 1% acetic acid, Sigma) were added to each well for 30 min. Finally, the plates were washed four times with 1% acetic acid (100 μL). The OD was measured at 540 nm using an ELISA reader (Bio-Rad). Paclitaxel was used as a positive control, whereas cells incubated only with 0.05% of DMSO were used as a negative control. All experiments were performed in triplicate.

ASSOCIATED CONTENT

Supporting Information Available:

The Supporting Information is available free of charge on the ACS website at DOI:

Selected NMR and mass spectra of compounds **1**, **2** and **3** and selected spectroscopic data of compounds **4**–**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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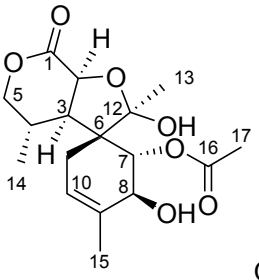

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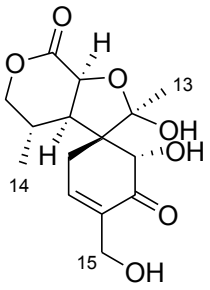

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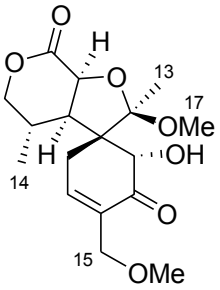

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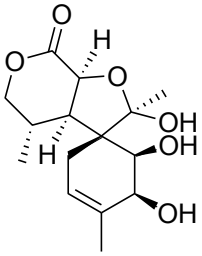

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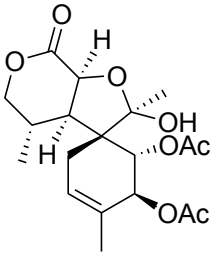

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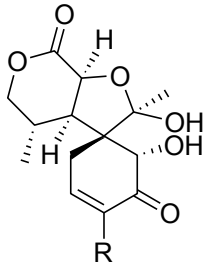

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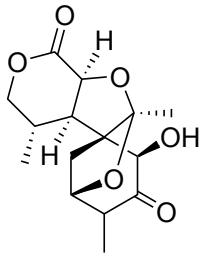

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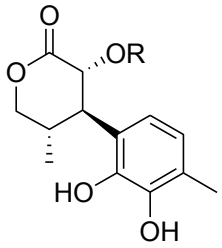


9: R = H
10: R = Ac

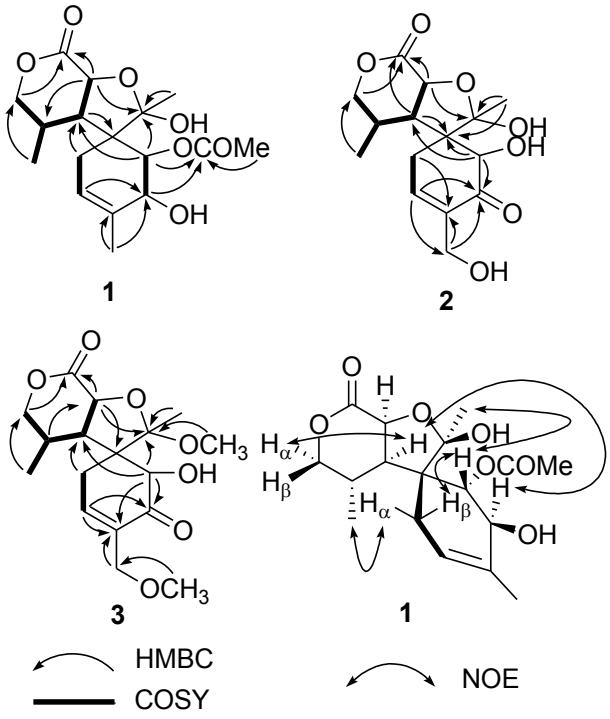


Figure 1. COSY, key HMBC, and key NOE correlations for **1**–**3**.

Table 1. ^{1}H NMR (600.22 MHz, 300 K) and ^{13}C NMR (151.91 MHz, 300 K) Data and ROESY Correlations for Compounds **1–3**.

	[a]8-deacetylcyclocalopin B (**1**)			[b]cyclocalopin A-15-ol (**2**)			[a]12,15-dimethoxycyclocalopin A (**3**)		
no.	δ_C, type	δ_H (*J* in Hz)	NOESY	δ_C, type	δ_H (*J* in Hz)	[a]ROESY	δ_C, type	δ_H (*J* in Hz)	ROESY
1	176.0, C			173.2, C			174.6, C		
2	73.5, CH	4.89 (d, 10.5)	3, 5α, 17	73.8, CH	4.56, (d, 10.0)	3, 5α	73.3, CH	4.74 (d, 10.3)	3, 5α
3	47.1, CH	2.77 (dd, 10.5, 10.5)	2, 5α, 8, 14	46.6, CH	2.19 (dd, 10.0, 10.0)	2, 5α ,14	45.4, CH	2.24 (dd, 10.3, 10.3)	2, 5α, 14
4	30.3, CH	2.23 (m)	5β, 14	30.8, CH	2.21 (m)	5β, 11α, 14	29.6, CH	2.21 (m)	5β, 11α, 14
5α	72.9, CH_2	4.05 (dd, 11.0, 11.0)	3, 5β, 14	72.5, CH_2	3.82 (dd, 11.0, 11.0)	2, 3, 5β, 14	72.6, CH_2	4.00 (dd, 11.2, 11.2)	2, 3, 5β, 14
5β		4.21 (m)	4, 5α, 14		4.07 (dd, 11.0, 1.6)	4, 5α, 14		4.18 (dd, 11.2, 3.2)	4, 5α, 14
6	52.9, C			56.5, C			56.7, C		
7	77.5, CH	5.07 (d, 9.4)	8, 11β, 13	76.3, CH	4.29 (s)	11β, 13	76.2, CH	4.63 (s)	11β, 13
8	71.9, CH	4.22 (d, 9.4)	3, 7, 15	199.3, C			200.4, C		
9	133.3, C			136.1, C			132.4, C		
10	123.1, CH	5.58 (m)	11α, 11β, 14, 15	145.9, CH	6.96 (m)	11α, 11β, 15	150.6, CH	7.18 (m)	11α, 11β, 15α, 15β
11α	27.2, CH_2	2.32 (dm, 20.2)	10, 11β, 14	28.0, CH_2	2.78 (dd, 21.3, 5.3)	4, 10, 11β, (14)	27.8, CH_2	2.85 (dd, 21.1, 6.2)	4, 10, 11β, 14
11β		2.18 (dm, 20.2)	7, 10, 11α, 13		2.55 (dm, 21.3)	7, 10, 11α, 13		2.69 (dm, 21.1)	7, 10, 11α, 13
12	108.3, C			107.6, C			110.9, C		
13	19.5, CH_3	1.45 (s)	7, 11β, 15	21.6, CH_3	1.61 (s)	7, 11β	13.9, CH_3	1.55 (s)	7, 11β, 17
14	14.6, CH_3	0.92 (d, 6.5)	3, 4, 5α, 5β, 10, 11α	16.8, CH_3	0.77 (d, 6.0)	3, 4, 5α, 5β, (11α)	15.5, CH_3	0.77 (d, 6.5)	3, 4, 5α, 5β, (11α)
15	17.5, CH_3	1.74 (s)	8, 10	60.2, CH_2	4.30 (m)	10	68.6, CH_2	4.08 (d, 12.3) 4.24 (d, 12.3)	10, 15β, 16 10, 15α, 16
16	173.9, C						57.7, OCH_3	3.36 (s)	15α, 15β
17	21.1, CH_3	2.14 (s)	2				48.4, OCH_3	3.17 (s)	13

Chemical shifts and coupling constants of **1**–**3** were determined in [a]D_2O and [b]$CDCl_3$+CD_3OD (10:1), respectively. Assignments were based on 2D NMR including HSQC and HMBC. Coupling constants were measured using ^{1}H NMR in combination with phase-sensitive COSY correlations. Well-resolved couplings are expressed with coupling patterns and coupling constants in Hertz (Hz) in parentheses.

Table 2. Anti-Staphylococcal Activity of the Compounds **1–10**.

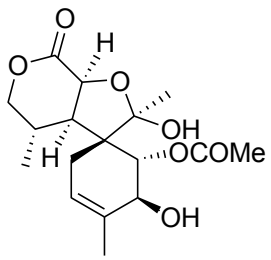
Compound	Microorganism, MIC [μg/mL]				
	ATCC 25923	XU212	SA-1199B	RN4220	EMRSA-15
1	16	32	16	ND	32
2	64	64	128	ND	128
3	128	128	256	ND	256
4	32	ND	64	ND	64
5	ND	64	64	ND	ND
6	32	ND	64	ND	64
7	64	128	64	ND	128
8	64	64	128	ND	64
9	64	128	128	ND	128
10	ND	64	128	ND	64
p. c.	32	32	64	ND	32

p. c. = Positive control (Norfloxacin). ND = Not detectable.

Table of Contents Graphic

Anti-Staphylococcal Calopins from Fruiting Bodies of *Caloboletus radicans*

Fakir Shahidullah Tareq, Choudhury Mahmood Hasan, M. Mukhlesur Rahman, Mohd Mukrish Mohd Hanafi, Lucio Colombi Ciacchi, Monika Michaelis, Tilmann Harder, Jan Tebben, Md. Tofazzal Islam, Peter Spiteller*



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