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Deconins A–E: Cuparenic and Mevalonic or Propionic Acid Conjugates from the Basidiomycete *Deconica* sp. 471

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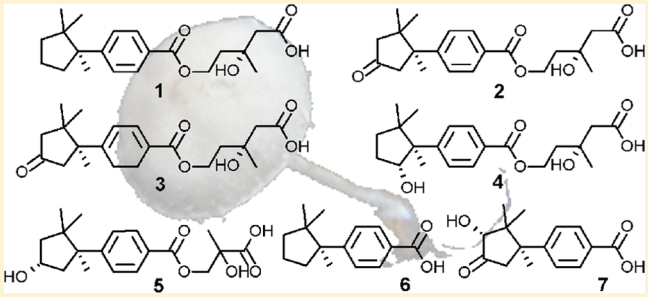
Supporting Information

ABSTRACT: Bioassay-guided fractionation of antibacterial extracts from cultures of a basidiomycete from Northern Thailand, which represents a new species of the genus *Deconica*, yielded the terpenoid deconin A (**1**), whose structure was elucidated by spectral methods (NMR, HRMS) as a cuparenic/mevalonic acid conjugate. The absolute configuration of **1** was determined after saponification and comparison of specific rotations of the resulting cuparenic acid and mevalonolactone with authentic standards and literature data. Six minor congeners (**2**–**7**) were isolated and identified, and their antimicrobial and cytotoxic effects are reported. Compounds **1**–**4** are the first natural products featuring an unmodified mevalonic acid residue as a building block.



Basidiomycetes have been found to produce a broad range of structurally diverse antibiotics. A fair example is the drug retapamulin, which was approved in 2007, but actually had been developed from pleuromutilin, a metabolite of *Clitopilus scyphoides* that had already been known for several decades.[1] Basidiomycetes are still underexploited compared to bacteria and ascomycetes,[2] and they constitute promising sources for the discovery of new antibiotics, which are urgently needed. We are presently focusing on antibiotics from Asian tropical basidiomycetes, which have been poorly investigated for both their secondary metabolite production and their taxonomy.[3,4]

Deconica sp. 471 was isolated from a fruiting body collected at Mt. Doi Mae Salong in Chiang Rai Province, Northern Thailand. Morphological studies and sequencing of the nucleotide sequences of 18S rDNA, internal transcribed spacers (ITS), and large subunit rRNA gene (LSU) indicated that strain 471 represents a new species in the genus *Deconica*.[5] This genus was established in 2009 to accommodate basidiomycetes of the family Strophariaceae that were formerly placed in the genus *Psilocybe* but do not produce hallucinogenic alkaloids of the psilocybin type and constitutes a yet widely unknown sister group of *Psilocybe* sensu stricto.[6] The crude extracts from submerged cultures of *Deconica* sp. 471 showed antimicrobial activities against Gram-positive bacteria, i.e., *Bacillus subtilis* and *Staphylococcus aureus*. Bioactivity-guided fractionation by RP-HPLC, using *B. subtilis* as the indicator organism, led to the isolation of the novel antimicrobial metabolite **1**, which constitutes the active principle.

Deconin A (**1**) (Chart 1) showed a molecular ion cluster $[M + H]^+$ at m/z 363.2166 in the HRESIMS, which provided the molecular formula $C_{21}H_{30}O_5$, implying 7 degrees of unsaturation. The ^{1}H NMR spectrum exhibited signals of two aromatic methines with dual intensity and six methylenes and four singlet methyl signals. The ^{13}C and DEPT spectra indicated 21 carbons, being two carbonyls, two aromatic methines (dual intensity), two aromatic quaternary carbons, six methylenes, four methyls, and three quaternary sp^3 hybridized carbon atoms. ^{1}H,^{1}H COSY and ^{1}H,^{13}C HMBC spectra indicated mevalonic acid and 4-(1,2,2-trimethylcyclopentyl)benzoic acid moieties (Figure 1). An HMBC correlation from H_2-5′ to C-1 explained the deep field shift of H_2-5′ and established the chemical structure. To reveal the absolute stereochemistry of compound **1**, the metabolite was saponified with sodium hydroxide, and the free cuparenic acid (**6**) was isolated subsequently by preparative HPLC. Its negative optical rotation indicated an *S* configuration opposite to its optical antipode (*R*)-(+)-cuparenic acid known from Cupressales.[7] In this study, the correlation of the absolute *R* configuration of cuparenic acid with a positive specific rotation had been demonstrated by total synthesis starting from (+)-cuparene. Furthermore, mevalonolactone, which spontaneously formed at pH 3,[8] was identified as *R* by GC/MS comparison with authentic standards.[9] Therefore, an 8*S*,3′*R* configuration was concluded for compound **1**.

In addition to **1**, a series of structurally related oxidized metabolites that differed in geometry and substitution pattern was isolated from crude extracts of multiple fermentations.

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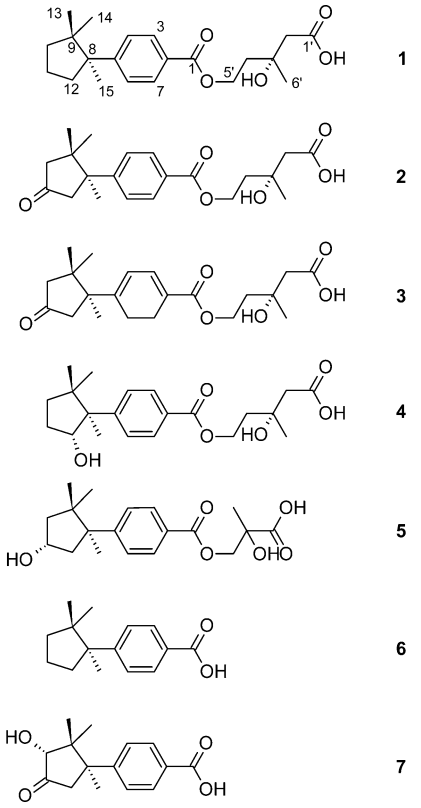
Chart 1. Metabolites Isolated from *Deconica* sp. 471



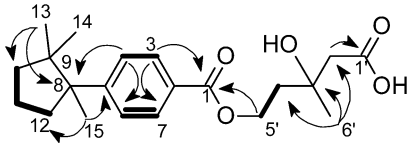


Figure 1. Key $^{1}H,^{1}H$ COSY (bold lines) and $^{1}H,^{13}C$ HMBC (arrows) correlations of deconin A (**1**).

Deconin B (**2**) was isolated as a colorless oil. Its molecular formula, $C_{21}H_{28}O_6$, was deduced from a prominent pseudomolecular ion cluster in its HRESIMS spectrum, indicating the formal substitution of two hydrogen atoms by an oxygen atom. The main difference in the ^{13}C and $^{1}H,^{13}C$ HSQC NMR spectra of **2** compared to **1** was the replacement of a methylene by a ketone group (δ_C 219.6). $^{1}H,^{13}C$ HMBC correlations from H_2-10 and H_2-12 located the ketone at C-11, characterizing **2** as the 11-oxo derivative of **1**.

The molecular formula $C_{21}H_{30}O_6$ of deconin C (**3**) was obtained by HRESIMS. The major difference in the NMR data of **3** compared to **2** was the presence of two additional methylenes (δ_H 2.42, 2.34) and the integral of only one proton for the olefinic protons H-3 and H-4. HMBC correlations from H_2-6 to C-4, C-5, and C-8 and from H_2-7 to C-1, C-2, and C-3 indicated the aromatic ring was reduced to a cyclohexadiene system. Therefore, **3** was identified as the 6,7-dihydro-11-oxo derivative of **1**.

Deconin D (**4**), a colorless oil, was identified as another $C_{21}H_{30}O_6$ isomer by HRESIMS. The location of the new hydroxy group was found at C-12 (δ_C 75.8) by COSY correlations from H_2-11 to H-12 and HMBC correlations from H_2-10, H_2-11, and H_3-15 to C-12. ROESY correlations between H-12 and H_3-13 and between H_3-14 and H_3-15 indicated a *cisoidal* arrangement between the C-12 hydroxy group and the H_3-15 methyl. Thus, **4** was established as the (*R*)-12-hydroxy derivative of **1**.

Deconin E (**5**), which was also obtained as a colorless oil, had a molecular formula of $C_{19}H_{26}O_6$ as determined by HRESIMS. Despite the absence of two carbon atoms, the NMR data pointed to a structural similarity to **4**. In addition to the shift of the hydroxy group to C-11, indicated by COSY correlations between H_2-10, H-11, and H_2-12, the key difference was the replacement of the mevalonic acid moiety by a 2,3-dihydroxy-2-methylpropionic acid unit. The stereochemistry of the sesquiterpenoid moiety was assigned from ROESY correlations: While H-11 correlated to H_b-10 and H_b-12, ROESY correlations were observed from H_3-15 to H_3-14 and H_a-12. Therefore, H_3-15 and the C-11 hydroxy group are *cisoidal* and an 11*R* configuration was concluded. The C-2′ configuration remained unassigned.

The molecular formula $C_{15}H_{20}O_2$ of metabolite **6** was provided by HRESIMS. ^{1}H and ^{13}C NMR resembled those of the cyclic part of deconin A (**1**); COSY and HMBC correlations identified cuparenic acid. The negative optical rotation indicated an *S* configuration and established the structure of **6** as *ent*-cuparenic acid.[7]

The molecular formula $C_{15}H_{18}O_4$ of **7** specified two additional oxygen atoms and an additional double-bond equivalent compared to **6**.[16] The ^{13}C NMR spectrum indicated the replacement of two methylene groups by a ketone and an oxymethine carbon. HMBC correlations from H_3-13/H_3-14 to oxymethine C-10 and H-10/H_2-12 to ketone C-11 identified **7** as 10-hydroxy-11-oxo-cuparenic acid. Finally, ROESY correlations between H-11 and H_3-13 and between H_3-14 and H_3-15 indicated a 10*R* configuration. Mosher's (MTPA) esters were synthesized for validation of the absolute configuration; the observed positive $\Delta\delta^{SR}$ chemical shift differences for H_3-13 and H_3-14 on one hand and the negative ones for H_a-12 and H_b-12 on the other confirm the 8*R*,10*R* configuration.

Cuparene-type sesquiterpenes (see Figure S2 in the Supporting Information for comparison) are widely distributed through all divisions of life, since they have been isolated from higher plants,[7] liverworts,[10] basidiomycete fungi,[11] and the sea hare *Aplysia dactyloma*.[12] Interestingly, from a biosynthetic and evolutionary point of view, some, like cuparene, exist as both enantiomers in nature, which is an uncommon phenomenon.[13] Whereas cuparenes from higher plants are regularly *R*, their *S* counterparts have been isolated from fungi and liverworts.[14] Consequently the *S*-chiralities of deconin and the *ent*-cuparenic acid derivatives are consistent. However, deconins A–E (**1**–**5**) are not simple sesquiterpenoids, but in **1**–**4** the sesquiterpene structure is extended by mevalonic acid to form the common scaffold. Although mevalonic acid is the precursor of all terpenes biosynthesized via the HMG-CoA reductase pathway, it has to the best of our knowledge never been observed as a substituent of another terpene skeleton. Deconins A–D (**1**–**4**) share this unprecedented structural entity.

The biological activity of metabolites **1**–**7** was assessed with a broad test panel of bacteria and fungi (see Table S1, Supporting Information). Deconin A (**1**) had weak activity (MIC = 67 μg/mL) against *Bacillus subtilis*, *Staphylococcus aureus*, and *Mucor hiemalis*. Cuparenic acid (**6**) had weak activity (MIC = 67 μg/mL) against *M. hiemalis*. Both deconins D (**4**) and E (**5**) showed weak activity (MIC = 67 μg/mL) against *S. aureus*. Furthermore, deconin D (**4**) also exhibited a moderate cytotoxic effect (IC_{50} = 7.4 μM) in a proliferation

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assay against the mouse fibroblast cell line L-929. Since these activities were in the same range as the crude extract; cumulative, synergistic effects of the deconins cannot be excluded.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined with a PerkinElmer 241 polarimeter; UV spectra were recorded with a Shimadzu UV−vis spectrophotometer UV-2450. NMR spectra were recorded with a Bruker 500 MHz Avance III spectrometer with a BBFO(plus) SmartProbe (^{1}H 500 MHz, ^{13}C 125 MHz) and a Bruker 700 MHz Avance III spectrometer with a 5 mm TCI cryoprobe (^{1}H 700 MHz, ^{13}C 175 MHz). HRESIMS mass spectra were obtained with an Agilent 1200 series HPLC-UV system combined with an ESI-TOF-MS (Maxis, Bruker) [column 2.1 × 50 mm, 1.7 μm, C_{18} Acquity UPLC BEH (Waters), solvent A: H_2O + 0.1% formic acid; solvent B: AcCN + 0.1% formic acid, gradient: 5% B for 0.5 min increasing to 100% B in 19.5 min, maintaining 100% B for 5 min, R_F = 0.6 mL min^{-1}, UV detection 200−600 nm].

Fermentation and Bioactivity-Guided Isolation of Deconin A (1). The mycelium from a cultivation in 1 L of YMG medium was separated from the culture broth by centrifugation (10 min at 1000*g*) and filtration. The wet mycelium was extracted twice with acetone (350 mL) in an ultrasonic bath at 30 °C for 30 min; the combined extracts were concentrated in vacuo at 40 °C. The remaining aqueous residue was diluted to 200 mL with water and extracted twice with 200 mL of ethyl acetate. The combined organic phases were dried over sodium sulfate and again evaporated in vacuo (40 °C) to dryness. The crude extract (ca. 50 mg) was dissolved in methanol. The crude extract was adsorbed through an RP-18 cartridge, eluted with methanol, and used for preparative HPLC as described below. The crude mycelial extract was fractionated by preparative RP-HPLC (column: 250 × 21 mm, VP Nucleodur C_{18} Gravity 7 μm, gradient: 40% to 100% acetonitrile with 0.5% acetic acid in 30 min, 100% for 15 min; flow rate: 15 mL/min). Fractions at t_R = 12−13 min were bioactive and contained 1.3 mg of **1**.

Fermentation and Isolation of Metabolites 2−7. Five small agar disks (ca. 1 cm diameter) with well-grown mycelia (10−12 days old) from agar plates (YM 6.3 medium) were used to inoculate 10 Erlenmeyer flasks (500 mL) containing 200 mL of YM 6.3 medium. The submerged cultures were incubated on a rotary shaker at 25 °C and 140 rpm. Samples were taken to determine the production of **1** by HPLC-MS during the fermentation; production started after 14 days and reached its maximum at 18 days. After the sugar was used up, the wet mycelium (ca. 68 g) was obtained as described above. The crude extract was filtered with methanol through an RP solid-phase cartridge (Strata-X 33 mm, Polymeric Reversed Phase; Phenomenex, Aschaffenburg, Germany) to yield 95 mg of crude product. The latter was divided into three portions and each subjected to preparative HPLC (PLC 2020, Gilson, Middleton, WI, USA). A VP Nucleodur C_{18} ec column (250 × 21 mm, 5 μm; Macherey-Nagel) was used as stationary phase. The mobile phase was composed of deionized water (Milli-Q, Millipore, Schwalbach, Germany) as solvent A and acetonitrile as solvent B. A flow rate of 15 mL min^{-1} was used for the following gradient: 10−50% solvent B in 30 min, 50−100% in 15 min, afterward isocratic conditions at 100% for 10 min. UV detection was carried out at 210 and 340 nm, and fractions were collected and combined according to the observed peaks. **2** (3.6 mg) was obtained at a retention time (t_R) = 32−32.5 min, **5** (0.8 mg) at t_R = 27.5−28 min, and a mixture of **2** and **4** (3.2 mg) at t_R = 32.5−33.5 min. From this mixture a final step of silica gel HPLC (column 250 × 21 mm, Nucleosil 100 7 μm, gradient from dichloromethane to dichloromethane/methanol (90:10) in 60 min, flow 20 mL/min) provided 1.2 mg of **2** (t_R = 9−12 min) and 1.2 mg of **4** (t_R 12−16 min).

Another cultivation of *Deconica* sp. 471 in 2 L scale was processed in the same way as described above. Parameters of the preparative RP-HPLC separation were the same as described above, but with a flow rate of 12 mL/min. **1** (1.0 mg) was obtained at t_R = 43−43.5 min, **3** (4.8 mg) at t_R = 32−32.5 min, **6** (0.4 mg) at t_R = 41−41.5 min, and **7** (1.4 mg) at t_R = 23.5−24 min.

Deconin A (1): colorless oil; $[\alpha]^{25}_D$ −22.4 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 244 (4.4) nm; IR (KBr) 3431, 2967, 2875, 1718, 1610, 1461, 1385, 1279, 1191, 1115, 1018, 776, 712 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 1; HRESIMS *m*/*z* 363.2166 ([M + H]$^+$, calcd for $C_{21}H_{31}O_5$, 361.2166).

Table 1. NMR Data (^{1}H 700 MHz, ^{13}C 175 MHz) of Deconin A (1) in Methanol-d4

pos	δ_C, type	δ_H, m (*J* in Hz)	HMBC
1	168.2, C		
2	128.7, C		
3/7	129.8, CH	7.97, d (8.6)	1, 5, 3/7
4/6	128.3, CH	7.53, d (8.6)	2, 4/6, 8
5	154.7, C		
8	52.1, C		
9	45.5, C		
10	40.8, CH_2	1.79, m	8, 9, 11, 12, 13, 14
		1.62, m	8, 9, 11, 12, 13, 14
11	20.6, CH_2	1.89, m	8, 9, 10, 12
12	37.9, CH_2	2.62, dt (12.8, 9.1)	5, 8, 11, 15
		1.79, m	5, 8, 9, 15
13	26.8, CH_3	0.60, s	8, 9, 10, 14
14	24.7, CH_3	1.15, s	8, 9, 10, 13
15	24.7, CH_3	1.35, s	5, 8, 9, 12
1′	175.8, C		
2′	47.1, CH_2	2.56, s	1′, 3′, 4′, 6′
3′	71.0, C		
4′	40.9, CH_2	2.16, dt (14.5, 6.7)	2′, 3′, 5′, 6′
		2.11, dt (14.5, 6.7)	
5′	62.5, CH_2	4.51, t (6.7)	3′, 4′, 1
6′	27.6, CH_3	1.41, s	2′, 3′, 4′

Deconin B (2): colorless oil; $[\alpha]^{25}_D$ −3.9 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 240 (4.4) nm; IR (KBr) 3445, 2970, 1718, 1610, 1460, 1407, 1281, 1191, 1116, 1017, 858, 777, 713 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.97 (2 H, d, *J* = 8.4 Hz, H-3, H-7), 7.53 (2 H, d, *J* = 8.4 Hz, H-4, H-6), 4.49 (2 H, t, *J* = 6.7 Hz, H_2-5′), 3.31 (1 H, d, *J* = 18.0 Hz, H_a-12), 2.57 (2 H, d, *J* = 1.8 Hz, H_2-2′), 2.44 (1 H, d, *J* = 18.9 Hz, H_a-10), 2.35 (1 H, d, *J* = 18.0 Hz, H_b-12), 2.23 (1 H, d, *J* = 18.9 Hz, H_b-10), 2.10 (2 H, m, H_2-4′), 1.46 (3 H, s, H_3-15), 1.38 (3 H, s, H_3-6′), 1.28 (3 H, s, H_3-14), 0.73 (3 H, s, H_3-13); ^{13}C NMR (CD_3OD, 125 MHz) δ 219.6 (C-11), 174.9 (C-1′), 167.9 (C-1), 151.4 (C-5), 130.3 (C-3, C-7), 129.6 (C-2), 128.2 (C-4, C-6), 71.0 (C-3′), 62.6 (C-5′), 53.2 (C-10), 51.4 (C-12), 50.7 (C-8), 46.8 (C-2′), 42.9 (C-9), 40.8 (C-4′), 27.6 (C-6′), 26.7 (C-13), 24.8 (C-15), 24.2 (C-14); HRESIMS *m*/*z* 377.1967 ([M + H]$^+$, calcd for $C_{21}H_{29}O_6$, 377.1959).

Deconin C (3): colorless oil; $[\alpha]^{25}_D$ −45 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 238 (4.4), 301 (4.1) nm; IR (KBr) 3440, 2963, 1726, 1610, 1574, 1461, 1405, 1384, 1280, 1224, 1123, 1117, 1017, 858, 777, 712 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.03 (1 H, dt, *J* = 6.1 Hz, 1.3 Hz, H-3), 5.98 (1 H, brd, *J* = 6.1 Hz, H-4), 4.32 (2 H, t, *J* = 6.7 Hz, H_2-5′), 2.95 (1 H, d, *J* = 18.0 Hz, H_a-12), 2.52 (2 H, d, *J* = 1.5 Hz, H_2-2′), 2.43 (1 H, m, H_2-7), 2.37 (1 H, m, H_2-6), 2.34 (1 H, m, H_a-10), 2.18 (1 H, m, H_b-10), 2.13 (1 H, d, *J* = 18.0 Hz, H_b-12), 2.00 (2 H, m, H_2-4′), 1.34 (3 H, s, H_3-6′), 1.24 (6 H, s, H_3-14, H_3-15), 1.04 (3 H, s, H_3-13); ^{13}C NMR (CD_3OD, 125 MHz) δ 219.6 (C-11), 174.9 (C-1′), 168.8 (C-1), 152.4 (C-10), 135.3 (C-3), 127.3 (C-2), 120.5 (C-4), 71.0 (C-3′), 62.5 (C-5′), 53.5 (C-10), 51.8 (C-12), 51.4 (C-8), 46.7 (C-2′), 42.3 (C-9), 40.7 (C-4′), 27.6 (C-6′), 26.9 (C-6), 26.6 (C-13), 25.0 (C-14) 22.9 (C-15), 22.6 (C-7); HRESIMS *m*/*z* 379.2119 ([M + H]$^+$, calcd for $C_{21}H_{31}O_6$, 379.2115).

Deconin D (4): colorless oil; $[\alpha]^{25}_D$ −0 (*c* 0.0, MeOH); UV (MeOH) λ_{max} (log ε) 244 nm; ^{1}H NMR (CD_3OD, 700 MHz) δ 7.96

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(2 H, d, J = 8.6 Hz, H-3, H-7), 7.55 (2 H, d, J = 8.6 Hz, H-4, H-6), 5.05 (1 H, t, J = 8.8 Hz, H-12), 4.48 (2 H, t, J = 6.7 Hz, H_2-5), 2.58 (2 H, brs, H_2-2), 2.19 (1 H, m, H_a-11), 2.11 (2 H, m, H_2-4′), 1.81 (1 H, m, H_a-10), 1.69 (1 H, m, H_b-11), 1.49 (1 H, m, H_b-10), 1.37 (3 H, s, H_3-6′), 1.28 (3 H, s, H_3-15), 1.08 (3 H, s, H_3-14), 0.51 (3 H, s, H_3-13); ^{13}C NMR (CD_3OD, 175 MHz) δ 175.5 (C-1′), 168.4 (C-1), 152.4 (C-5), 130.1 (C-3, C-7), 129.0 (C-2), 128.7 (C-4, C-6), 75.8 (C-12), 71.0 (C-3′), 62.5 (C-5′), 54.7 (C-8), 47.1 (C-2), 44.4 (C-9), 41.0 (C-4′), 37.3 (C-10), 29.8 (C-11), 28.4 (C-13), 27.7 (C-6′), 25.6 (C-14), 17.2 (C-15); HRESIMS m/z 379.2121 ([M + H]$^+$, calcd for $C_{21}H_{31}O_6$, 379.2115).

*Deconin E (**5**):* colorless oil; $[\alpha]^{25}_D$ +1.5 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 240 (4.2) nm; IR (KBr) 3415, 2961, 1721, 1610, 1460, 1384, 1278, 1193, 1123, 1019 cm^{-1}; ^{1}H NMR (CD_3OD, 700 MHz) δ 7.95 (2 H, d, J = 8.6 Hz, H-3, H-7), 7.46 (2 H, d, J = 8.6 Hz, H-4, H-6), 4.53 (1 H, dddd, J = 8.6 Hz, 8.0 Hz, 4.7 Hz, 2.6 Hz, H-11), 4.45 (1 H, d, J = 10.8 Hz, H_a-3), 4.38 (1 H, d, J = 10.8 Hz, H_b-3), 3.00 (1 H, dd, J = 14.0 Hz, 8.6 Hz, H_a-12), 2.00 (1 H, dd, J = 13.6 Hz, 8.0 Hz, H_a-10), 1.81 (1 H, dd, J = 13.6 Hz, 4.7 Hz, H_b-10), 1.78 (1 H, dd, J = 14.0 Hz, 2.6 Hz, H_b-12), 1.49 (3 H, s, H_3-4′), 1.47 (3 H, s, H_3-15), 1.19 (3 H, s, H_3-14), 0.54 (3 H, s, H_3-13); ^{13}C NMR δ, 177.2 (C-1′), 167.6 (C-1), 154.4 (C-5), 130.1 (C-3, C-7), 128.4 (C-2), 128.2 (C-4, C-6), 74.7 (C-2′), 70.9 (C-3′), 70.5 (C-11), 52.1 (C-8), 51.5 (C-10), 48.0 (C-12), 46.0 (C-9), 27.0 (C-13), 26.1 (C-15), 24.9 (C-14), 23.0 (C-4′); HRESIMS m/z 351.1805 ([M + H]$^+$, calcd for $C_{19}H_{27}O_6$, 351.1802).

*ent-Cuparenic acid (**6**):* colorless oil; $[\alpha]^{25}_D$ −120 (c 0.03, MeOH); ^{1}H NMR (CD_3OD, 700 MHz) δ 7.88 (2 H, d, J = 8.2 Hz, H-3, H-7), 7.42 (2 H, d, J = 8.2 Hz, H-4, H-6), 2.58 (1 H, m, H_a-12), 1.84 (2 H, m, H-11), 1.74 (2 H, m, H_a-10, H_b-12), 1.58 (1 H, s, H_b-10), 1.31 (3 H, s, H_3-15), 1.10 (3 H, s, H_3-14), 0.56 (3 H, s, H_3-13); ^{13}C NMR (CD_3OD, 175 MHz) δ, 167.9 (C-1), 154.9 (C-5), 130.0 (C-3, C-7), 128.4 (C-2), 128.3 (C-4, C-6), 52.2 (C-8), 45.5 (C-9), 40.8 (C-10), 37.9 (C-12), 26.8 (C-13), 24.7 (C-14, C15), 20.6 (C-11); HRESIMS m/z 233.1542 ([M + H]$^+$, calcd for $C_{15}H_{21}O_2$, 233.1536).

*(8R,10R)-10-Hydroxy-11-oxo-cuparenic acid (**7**):* colorless oil; $[\alpha]^{25}_D$ +3 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 235 (3.8) nm; IR (KBr) 3435, 2972, 1745, 1705, 1611, 1410, 1384, 1272, 1196, 1080, cm^{-1}; ^{1}H NMR (CD_3OD, 700 MHz) δ 7.97 (2 H, d, J = 8.6 Hz, H-3, H-7), 7.41 (2 H, d, J = 8.2 Hz, H-4, H-6), 3.95 (1 H, d, J = 1.7 Hz, H-10), 3.04 (1 H, dd, J = 19.8 Hz, 1.7 Hz, H_a-12), 2.47 (1 H, d, J = 19.8 Hz, H_b-12), 1.56 (3 H, s, H_3-15), 1.00 (3 H, s, H_3-14), 0.79 (3 H, s, H_3-13); ^{13}C NMR (CD_3OD, 175 MHz) δ 218.3 (C-11), 169.6 (C-1), 152.9 (C-5), 130.2 (C-3, C-7), 129.9 (C-2), 128.9 (C-4, C-6), 82.5 (C-10), 48.6 (C-12), 46.3 (C-9), 45.4 (C-8), 24.5 (C-15), 23.3 (C-13), 18.1 (C-14); HRESIMS m/z 263.1282 ([M + H]$^+$, calcd for $C_{15}H_{19}O_4$, 263.1278).

Minimum Inhibitory Concentrations. MICs were determined (Table S1) in 96-well microtiter plates in a serial dilution assay with EBS medium (0.5% peptone [Marcor], 0.5% glucose, 0.1% meat extract, 0.1% yeast extract, 50 mM HEPES [11.9 g/L], pH 7.0) for bacteria and MYC medium (1.0% phytone peptone, 1.0% glucose, 50 mM HEPES [11.9 g/L], pH 7.0) for yeasts and fungi, respectively.[15] First, a 20 μL stock solution of each compound at 1 mg/mL in MeOH (2 μL of reference drugs) was pipetted into the first row (A) of the plate, and a few minutes allowed for the solvents to evaporate. Negative control wells were left blank. Next, 150 μL of a mixture of the test pathogen and the culture medium in the ratio of 1:100, respectively, was aliquoted in all the rows. To the first row was added an additional 150 μL of the pathogen−medium mixture and mixed by repeated pipetting, before transferring 150 μL of this mixture to the second row. A 1:1 serial dilution was done in the subsequent rows, and 150 μL discarded after the last row (H). Plates were incubated on a microplate vibrating shaker (Heidolph Titramax 1000) at 600 rpm at 30 °C for 24−48 h. The lowest concentration of the compounds preventing visible growth of the pathogen was taken as the MIC. The concentrations tested ranged from 66.7 to 0.1 μg/mL.

Cytotoxicity Assay. *In vitro* cytotoxicity (IC_{50}) was determined against mouse fibroblast cell line L929 cultured in EBM-2 (Lonza) supplemented with 10% fetal bovine serum (Gibco) under 10% CO_2 at 37 °C. A 60 μL amount of serial dilutions from an initial stock of 1 mg/mL in MeOH of the test compounds was added to 120 μL aliquots of a cell suspension (50 000/mL) in 96-well microplates. After 5 days of incubation, an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed, and the absorbance measured at 590 nm using an ELISA plate reader (Victor). The concentration, at which the growth of cells was inhibited to 50% of the control (IC_{50}), was obtained from the dose−response curves. The negative control was methanol.

Absolute Configuration of 1. Deconin A (**1**, 0.8 mg) was hydrolyzed with 0.1 M NaOH (500 μL) for 16 h at 25 °C. The solution was neutralized with 1 M HCl (50 μL). Analysis by ESIMS revealed the complete hydrolysis of **1**. The mixture was fractionized by RPHPLC; a VP Nucleodur C_{18} ec column (250 × 21 mm, 5 μm; Macherey-Nagel) was used as stationary phase. The mobile phase was composed of deionized water (Milli-Q, Millipore, Schwalbach, Germany) as solvent A and acetonitrile as solvent B, both with 0.05% TFA. A flow rate of 12 mL min^{-1} was used with the gradient from 40−100% solvent B in 30 min; *ent*-cuparenic acid (**6**, 0.3 mg) eluted at 23.5−24 min.

All other fractions were combined, concentrated in vacuo, and acidified to pH 3 with formic acid. The solution (100 mL) was extracted with CH_2Cl_2 (100 mL) for 16 h. The CH_2Cl_2 extract was dried (Na_2SO_4) and analyzed by GC-MS. Retention times of commercial *R*- and *S*-mevalonolactone were compared with the obtained material on Chirasil-Val.

Synthesis of (*R*)- and (*S*)-MTPA Esters of 7. For the preparation of the (*R*)-MTPA ester 0.15 mg of **7** was dissolved in 600 μL of pyridine-d_5, and 7 μL of (*S*)-MTPA chloride was added. The mixture was kept at 25 °C for 15 min before measurement of proton NMR spectra: ^{1}H NMR (pyridine-d_5, 700 MHz, only relevant signals assigned) δ 0.78 (s, H_3-13), 0.97 (s, H_3-14), 1.44 (s, H_3-15), 2.73 (d, J = 19.9 Hz, H_b-12), 3.32 (d, J = 19.9 Hz, H_a-12). The (*S*)-MTPA ester was prepared in the same manner by the addition of 7 μL of (*R*)-MTPA chloride: ^{1}H NMR (pyridine-d_5, 700 MHz, only relevant signals assigned) δ 0.86 (s, H_3-13), 1.09 (s, H_3-14), 1.46 (s, H_3-15), 2.69 (d, J = 19.9 Hz, H_b-12), 3.29 (d, J = 19.9 Hz, H_a-12).

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, NMR and mass spectra, morphological and phylogenetic details of the producing organism. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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