The Journal of Antibiotics (2018) 71:351–358
https://doi.org/10.1038/s41429-017-0008-9

JARA Japan Antibiotics Research Association
The Society for Actinomycetes Japan

ARTICLE

Cordybislactone, a stereoisomer of the 14-membered bislactone clonostachydiol, from the hopper pathogenic fungus *Cordyceps* sp. BCC 49294: revision of the absolute configuration of clonostachydiol

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Received: 6 September 2017 / Revised: 16 November 2017 / Accepted: 22 November 2017 / Published online: 18 January 2018

Abstract

Cordybislactone (**3**), a new stereoisomer of the 14-membered bislactone clonostachydiol, together with its open ring analog (**4**), was isolated from the hopper pathogenic fungus *Cordyceps* sp. BCC 49294. The relative and absolute configurations of **3** were determined by chemical derivatizations, including the modified Mosher's method. The stereochemistry of clonostachydiol was determined using the natural compound isolated from *Xylaria* sp. BCC 4297. The result revealed that the absolute configuration of clonostachydiol, previously determined by synthesis, should be revised to its enantiomer.

Introduction

The isolation of clonostachydiol, an anthelmintic 14-membered bislactone, from the fungus *Clonostachys cylindrospora* (strain FH-A 6607) was reported in 1993 by Zeeck and co-workers [1, 2]. The planar structure was presented in the original isolation paper. Its stereochemistry was determined in 1995 by asymmetric synthesis as to be **1a** (Fig. 1) by Rama Rao and co-workers [3, 4]. Many years later, the second and third asymmetric synthesis of clonostachydiol had been reported by independent research groups [5, 6]. A confusing issue was that the ^{1}H NMR spectroscopic data in DMSO-d_6 are apparently different between those in original isolation paper [1]/patent [2] and those in synthesis papers [3, 6]. Clonostachydiol was also isolated from *Gliocladium* sp [7]. and *Xylaria obovata* ADA-228 [8]. In both reports, NMR data are not presented as it was handled as a known compound. We also isolated this compound from *Xylaria* sp. BCC 4297 [9]. The ^{1}H NMR spectrum in DMSO-d_6 matched with the synthetic compound [3], and the optical rotation data of our sample was consistent with both reported data for the natural product [1, 2, 7] ($[\alpha]^{20}_{D} + 103$, *c* 1.0, MeOH; original isolation paper) and synthetic samples [3, 5, 6]. From *Gliocladium* sp., the C-4 ketone derivative, named 4-keto-clonostachydiol, was also isolated along with clonostachydiol by Munro and co-workers [7]. They performed chemical correlation: $NaBH_4/CeCl_3$ reduction of the ketone to give clonostachydiol and its C-4 epimer. On the basis of the optical rotation data of the reduction product, the authors assigned the stereochemistry of the natural products from the fungus as **1a** and **2a**. Recently, She and co-workers reported asymmetric synthesis of 4-keto-clonostachydiol, both the reported structure **2a** and its enantiomer **2b** [10]. On the basis of the optical rotation data, they concluded that the absolute configuration of natural 4-keto-clonostachydiol (from *Gliocladium* sp.) needs to be revised to be **2b**. She's group did not perform chemical correlation to clonostachydiol. However, taking together with the works by Munro's group [7], it leads to the conclusion that natural clonostachydiol should be **1b**. This is inconsistent with the reports of asymmetric synthesis by three independent Indian research groups (**1a**), therefore, either one should be wrong.

In our continuing search for novel bioactive compounds from invertebrate pathogenic fungi, a stereoisomer of

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41429-017-0008-9) contains supplementary material, which is available to authorized users.

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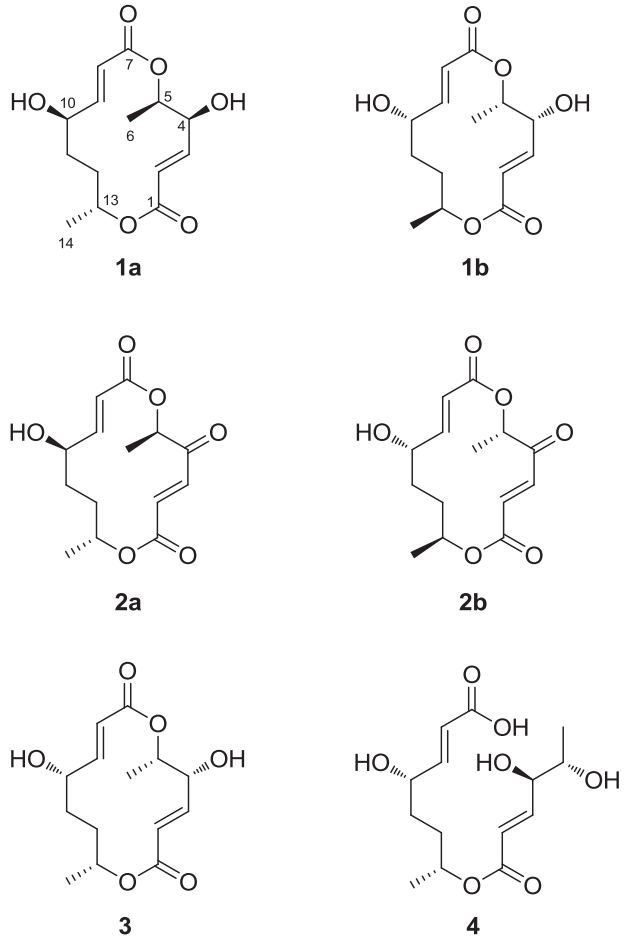


Fig. 1 Structures of macrobislactones

clonostachydiol, cordybislactone (**3**), and its hydrolyzed derivative (**4**), have been isolated from cultures of the hopper pathogen, *Cordyceps* sp. BCC 49294. In this paper we describe the isolation and structure elucidation of these new compounds. In addition, for solving above mentioned queries on the stereochemistry of clonostachydiol, we have conducted re-isolation of the "presumed" clonostachydiol from *Xylaria* sp. BCC 4297, and determined its absolute configuration.

Results and Discussion

Cordybislactone (**3**) was isolated as a colorless amorphous solid, and the molecular formula was determined as $C_{14}H_{20}O_6$ by HRESIMS. The ^{1}H and ^{13}C NMR, DEPT135, and HSQC spectroscopic data indicated the presence of 14 carbons categorized as two ester carbonyl carbons, four sp^2 methines composing two *trans* olefins, four oxygenated methines, two methylenes, and two methyl groups (Table 1). In addition, the ^{1}H NMR spectrum exhibited resonances of two hydroxyl groups. The planar structure, same as clonostachydiol, was elucidated on the basis of COSY and HMBC correlations. Thus, two ester linkages constructing a bislactone was indicated by HMBC correlations from H-5, H-8, and H-9 to C-7 (δ_C 165.0), and from H-2, H-3, and H-13 to C-1 (δ_C 165.6).

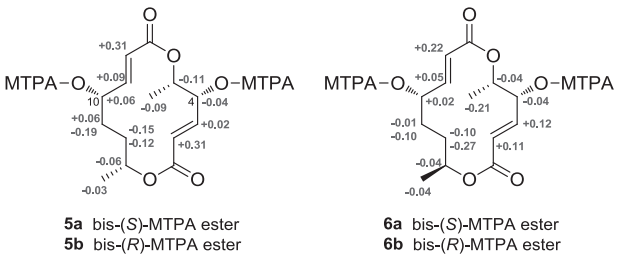
The molecular formula of **4** was determined by HRESIMS as $C_{14}H_{22}O_7$, a (partial) hydrolysate of **3**. The significant difference of the NMR spectra was the upfield shift of H-5 (δ_H 3.75, m; in acetone-d_6) when compared with **3** (H-5, δ_H 5.03, m; in acetone-d_6). The ester linkage involving C-1 (δ_C 166.4) was indicated by HMBC correlations from H-2, H-3, and H-13 to the carbonyl carbon. Consequently, compound **4** was identified as the C-7 ester hydrolyzed derivative. It was further confirmed by conversion of **3** into this compound. Treatment of **3** with 0.4 M aqueous K_2CO_3/dioxane at room temperature gave **4** as the major product.

Clonostachydiol was re-isolated [9] from *Xylaria* sp. BCC 4297. Its specific rotation, $[\alpha]^{23}_D + 102$ (*c* 1.05, MeOH), was consistent with all reported data, both isolation and synthesis. NMR spectra were taken in four different solvents, DMSO-d_6, $CDCl_3$, acetone-d_6, and CD_3OD. Confusingly, the ^{1}H NMR data reported in the original isolation paper (from *Clonostachys cylindrospora* strain FH-A 6607, in DMSO-d_6) were consistent with those of the compound from BCC 4297 taken in $CDCl_3$, while the ^{13}C NMR data of the original paper (in DMSO-d_6) matched well with the data of our isolated compound taken in DMSO-d_6. On the other hand, ^{1}H NMR data of the synthetic compound (first and third total synthesis) [3, 6] in DMSO-d_6 were consistent with those of our isolated compound in DMSO-d_6. On the basis of these results, it was concluded that wrong ^{1}H NMR solvent was recorded in the original isolation paper [1] and corresponding patent [2]. It should also be noted that the ^{1}H NMR spectrum of the second total synthesis compound (Yadav et al.) was taken in $CDCl_3$ [5], and the recorded data were different from any of our ^{1}H NMR spectra of clonostachydiol (four solvents). It suggested that the synthetic compound may not be clonostachydiol. Consequently, the remained important question was whether natural clonostachydiol is **1a** or **1b**.

With enough quantities of samples for derivatizations in hand, absolute configurations of cordybislactone (**3**, from BCC 49294) and clonostachydiol (from BCC 4297) were determined. The absolute configurations of the secondary alcohol carbons, C-4 and C-10, were determined by application of the modified Mosher's method [11]. Thus, cordybislactone (**3**) was acylated with (*R*)- and (*S*)-MTPA-Cl to obtain bis-(*S*)- and bis-(*R*)-MTPA ester derivatives **5a** and **5b**, respectively. The $\Delta\delta$-values indicated the 4*R*,10*S* configuration of **3** (Fig. 2). However, there was only one small inconsistency with the rule of the sign of $\Delta\delta$, + 0.06 for H_a-11. For application of the modified Mosher's method

Table 1 NMR spectroscopic data for cordybislactone (3, isolated from *Cordyceps* sp. BCC 49294) and clonostachydiol (1b, isolated from *Xylaria* sp. BCC 4297)

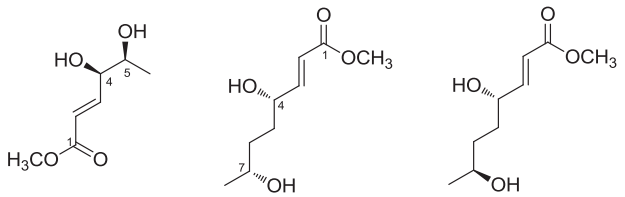
position	3 (DMSO-d_6, 500 MHz)		**1b** (DMSO-d_6, 500 MHz)		**1b** ($CDCl_3$, 500 MHz)	
	δ_C, mult.	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)
1	165.6, C		164.8		165.0	
2	122.8, CH	5.98, d (15.6)	123.5	5.92, d (15.7)	123.9	5.97, d (15.7)
3	147.0, CH	6.70, dd (15.6, 6.2)	147.6	6.48, dd (15.7, 8.7)	145.3	6.75, dd (15.7, 6.3)
4	73.6, CH	4.17, m	75.0	3.96, ddd (14.7, 8.7, 5.9)	76.6	4.16, m
5	73.4, CH	4.89, m	71.3	4.73, dq (14.7, 6.3)	73.8	5.03, m
6	17.8, CH_3	1.29, d (6.5)	17.6	1.33, d (6.3)	18.0	1.48, d (6.5)
7	165.0, C		165.0		166.4	
8	118.8, CH	5.81, dd (15.5, 1.5)	120.0	5.78, dd (15.8, 1.3)	120.3	5.94, dd (15.9)
9	152.2, CH	6.71, dd (15.5, 2.8)	153.0	6.67, dd (15.8, 4.1)	150.8	6.89, dd (15.9, 3.8)
10	68.3, CH	4.17, m	68.2	4.46, m	70.4	4.50, m
11	30.3, CH_2	1.70, m; 1.44, m	28.2	1.82, m; 1.57, m	30.6	1.88, m; 1.79, m
12	27.9, CH_2	1.57, m; 1.47, m	25.7	1.50–1.39, m	27.4	1.76, m; 1.55, m
13	70.2, CH	4.95, m	69.2	5.02, m	70.1	5.16, m
14	18.9, CH_3	1.16, d (6.4)	17.3	1.13, d (6.6)	18.7	1.23, d (6.5)
4-O*H*		5.57, d (4.7)		5.71, d (5.9)		
10-O*H*		5.16, d (4.3)		5.07, d (3.9)		



5a bis-(*S*)-MTPA ester
5b bis-(*R*)-MTPA ester

6a bis-(*S*)-MTPA ester
6b bis-(*R*)-MTPA ester

Fig. 2 $\Delta\delta$-Values (δ_S–δ_R) of the bis-MTPA esters **5a/5b** and **6a/6b** (Color figure online)



7 **8** **9**

Fig. 3 Structures of the methanolysis products

of a linear mono secondary alcohol (typical case), it is generally requested to perfectly match with the $\Delta\delta$-value rule. As for **3**, the small mismatch is not so surprising, since **5a/5b** are bis-MTPA esters of a macrocyclic diol. Similarly, clonostachydiol was also converted into bis-MTPA esters **6a** and **6b**, whose ^{1}H NMR spectroscopic data unambiguously revealed the 4 *R*,10 *S* configuration.

Cleavage of the two ester bonds of **3** was achieved by treatment with NaOMe in dry MeOH to furnish methyl ester fragments **7** and **8** (Fig. 3). Similarly, methanolysis of clonostachydiol gave the fragments **7** and **9**. The shorter diol (**7**) was identical to that from **3** (^{1}H NMR). Preparation of the acetonide derivative of **7** met with failure. However, selective methanolysis of **3** (K_2CO_3, MeOH) and subsequent treatment of the mono-methanolysis product with *p*-TsOH·H_2O in 2,2-dimethoxypropane gave an acetonide derivative **10** (72%, over 2 steps). The NOESY correlations of **10** (Fig. 4) indicated that it is a *cis* acetonide. Taking together with the 4 *R* configuration as described above, both **3** and clonostachydiol were shown to possess 4 *R*,5 *S* configuration.

The only remained question was the absolute configuration of C-13 for **3** and clonostachydiol, which was also deduced by application of the modified Mosher's method for the diol fragments **8** and **9** (Fig. 5). Since their 4 *S* configuration (corresponding to C-10 of **3** and clonostachydiol) has already been established, one of these diols should have 4 *S*,7 *R* configuration, while the other should be the 4 *S*,7 *S* isomer. Diol **8**, the longer fragment of **3**, was derivatized with Mosher reagents to afford bis-(*S*)- and bis-(*R*)-MTPA esters **11a** and **11b**, respectively. The $\Delta\delta$-values were consistent with the 7 *R* isomer (corresponding to 13 *R* configuration of **3**), showing positive sign $\Delta\delta$ for H_3-8, and negative sign large $\Delta\delta$-values for the four methylene protons H_2-5 and H_2-6. This result, in turn, demonstrated the 4 *S*,7 *S* configuration of the other diol **9**. To further support this conclusion, bis-(*S*)- and bis-(*R*)-MTPA esters **12a** and **12b** were synthesized from **9**. The $\Delta\delta$-values, in particular,

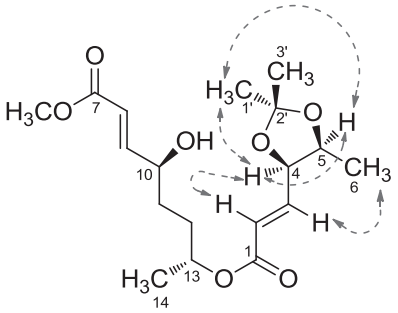


Fig. 4 Key NOESY correlations of the acetonide **10** (Color figure online)

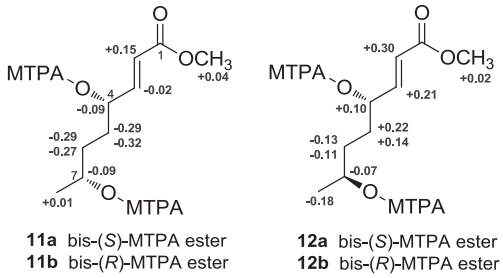


Fig. 5 $\Delta\delta$-Values (δ_S–δ_R) of the bis-MTPA esters **11a/11b** and **12a/12b** (Color figure online)

negative sign large $\Delta\delta$-value for H_3-8 (-0.18) and positive sign $\Delta\delta$-value for H-4 ($+0.10$) were consistent with the 7 *S* configuration (corresponding to 13 *S* configuration of clonostachydiol). Because the 4-O-MTPA group should contribute to the negative sign $\Delta\delta$ for H_2-5 and H_2-6, the large chemical shift effects resulting positive sign $\Delta\delta$-values for H_2-5 should be the major contribution of the 7-O-MTPA group and the data suggested 7 *S* configuration. There are often cases in the Mosher method analysis that chemical shift effects (shielding by the phenyl group of MTPA) for β-position protons of a secondary alcohol (Mosher ester) are larger than those of α-position protons, especially when a linear carbon chain preferably adopt zigzag conformations. Therefore, the $\Delta\delta$-values for the four methylene protons H_2-5 and H_2-6 are also in good agreement with the 4 *S*,7 *S* configuration. It should also be noted that the reversed configurational assignment between **8** and **9** (4 *S*,7 *S* for **8**; 4 *S*,7 *R* for **9**) is totally inconsistent with the Mosher method rule. Taking all results together, the absolute configuration of cordybislactone (**3**) was determined to be 4 *R*,5 *S*,10 *S*,13 *R*. Clonostachydiol was identified to be the 4 *R*,5 *S*,10 *S*,13 *S* isomer **1b**, which is the enantiomer of the previously reported structure (**1a**).

Our conclusion is consistent with the report of She's group [10], and we propose here the revision of the previously reported absolute configuration of clonostachydiol. Natural clonostachydiol, isolated from *Clonostachys cylindrospora* (strain FH-A 6607), *Gliocladium* sp., and *Xylaria* sp. BCC 4297, all showing positive sign optical rotation, should be **1b**.

Clonostachydiol is reported to exhibit no significant activity in antibacterial, antifungal, antiviral, and antiprotozoal assays, while it was shown to possess weak cytostatic activity and anthelmintic activity [1]. Compounds **3** and **1b** were subjected to several biological assays: cytotoxicity to tumor cell-lines (KB, NCI-H187, and MCF-7), antimycobacterial activity (*Mycobacterium tuberculosis* H37Ra), and antibacterial activity (*Bacillus cereus*, *Enterococcus faecium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*). Cordybislactone (**3**) showed weak cytotoxicity: KB, IC_{50} 43 μg/ml; NCI-H187, IC_{50} 14 μg/ml; MCF-7, $IC_{50} > 50$ μg/ml. These results were similar to those of clonostachydiol (**1b**): KB, IC_{50} 39 μg/ml; NCI-H187, IC_{50} 17 μg/ml; MCF-7, $IC_{50} > 50$ μg/ml. Both compounds showed week antimycobacterial activity with the same MIC of 50 μg/ml, while they were inactive in all antibacterial assays.

Methods

General experimental procedures

Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on an Analytik-jena SPEKOL 1200 spectrophotometer. FTIR spectra were taken on a Bruker ALPHA spectrometer. NMR spectra were recorded on Bruker DRX400 and AV500D spectrometers. ESITOF mass spectra were measured with a Bruker micrOTOF mass spectrometer.

Fungal material

Cordyceps sp. (Cordycipitaceae) was isolated from an unidentified hopper (Hemiptera) collected in Chiang Dao Wildlife Sanctuary, Chiang Mai Province, Thailand, on August 17, 2011, and it was deposited in the BIOTEC Culture Collection as BCC 49294. Identification of this fungus is based on the morphology and ITS rDNA sequence data (GenBank accession number: KT919971). *Xylaria* sp. (Xylariaceae) was isolated from an unidentified dead wood in Hala Bala Wildlife Sanctuary, Narathiwat Province, Thailand, and it was deposited in the BIOTEC Culture Collection as BCC 4297 on February 24, 2000. Original identification based on the morphology was later confirmed by the ITS5-4 rDNA sequence data (GenBank accession number: MF784453).

Fermentation, extraction, and isolation: Cordyceps sp. BCC 49294

The fungus BCC 49294 was fermented in 60 × 1000 ml Erlenmeyer flasks containing 250 ml of M102 medium (sucrose 30 gl^{-1}, malt extract 20 gl^{-1}, Bacto-peptone 2.0 gl^{-1}, yeast extract 1.0 gl^{-1}, KCl 0.5 gl^{-1}, $MgSO_4{\cdot}7H_2O$ 0.5 g, KH_2PO_4 0.5 gl^{-1}) at 25 °C for 26 days under static conditions. The cultures were filtered, and the filtrate (broth) was extracted with EtOAc (15 l × 3). The organic layers were concentrated under reduced pressure to obtain a brown gum (10.16 g). This broth extract was passed through a Sephadex LH-20 column chromatography (CC) (4.0 × 40 cm) eluted with MeOH to obtain five pooled fractions (Fr-1 – Fr-5), where the major fraction, Fr-3 (8.93 g) contained polyketide metabolites. Fr-3 was subjected to silica gel CC (6.5 × 16 cm, MeOH/CH_2Cl_2, step gradient elution from 0:100 to 14:86) and the fractions were further purified by silica gel CC (MeOH/CH_2Cl_2) to furnish **3** (4.87 g) and **4** (15.6 mg).

*Cordybislactone (**3**)*: colorless gum; $[\alpha]^{24}{}_D + 153$ (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 217 (3.57) nm; IR (ATR) ν_{max} 3422, 1704, 1655, 1275 cm^{-1}; ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data in DMSO-d_6, see Table 1; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data in acetone-d_6, see Supplementary Information (Supplementary Table S1); HRMS (ESI-TOF) *m/z* 307.1157 $[M + Na]^+$ (calculated for $C_{14}H_{20}O_6Na$, 307.1152).

Compound **4**: colorless gum; $[\alpha]^{26}{}_D + 4$ (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 217 (3.68) nm; IR (ATR) ν_{max} 2923, 2852, 1704, 1278, 1179 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 7.05 (1 H, dd, $J = 15.7$, 4.6 Hz, H-3), 6.92 (1 H, dd, $J = 15.6$, 4.6 Hz, H-9), 6.06 (1 H, dd, $J = 15.7$, 1.7 Hz, H-2), 6.01 (1 H, dd, $J = 15.6$, 1.5 Hz, H-8), 4.97 (1 H, m, H-13), 4.33 (1 H, m, H-10), 4.14 (1 H, m, H-4), 3.75 (1 H, m, H-5), 1.76 (1 H, m, H_a-11), 1.66 (1 H, m, H_b-11), 1.64-1.57 (2 H, m, H-12), 1.23 (3 H, d, $J = 6.3$ Hz, H-14), 1.14 (3 H, d, $J = 6.3$ Hz, H-6); ^{13}C NMR (125 MHz, acetone-d_6) δ 167.7 (C, C-7), 166.4 (C, C-1), 152.3 (CH. C-9), 149.1 (CH, C-3), 122.0 (CH, C-2), 120.5 (CH, C-8), 75.6 (CH, C-4), 71.0 (CH, C-14), 70.7 (CH, C-5), 70.4 (CH, C-10), 33.1 (CH_2, C-12), 32.4 (CH_2, C-11), 20.3 (CH_3, C-14), 19.0 (CH_3, C-6); HRMS (ESI-TOF) *m/z* 325.1261 $[M + Na]^+$ (calculated for $C_{14}H_{22}O_7Na$, 325.1258).

Fermentation, extraction, and isolation: Xylaria sp. BCC 4297

The fermentation of the fungus BCC 4297 was conducted using a similar procedure as previously reported [9]. Final fermentation was carried out in 60 × 1000 ml Erlenmeyer flasks containing 250 ml of malt extract broth (MEB; malt extract 6.0 gl^{-1}, yeast extract 1.2 gl^{-1}, maltose 1.8 gl^{-1}, dextrose 6.0 gl^{-1}) at 25 °C for 54 days under static conditions. The cultures were filtered, and the filtrate (broth) was extracted with EtOAc (13 l × 3). The organic layers were concentrated under reduced pressure to obtain a brown gum (4.47 g). This broth extract was passed through a Sephadex LH-20 column chromatography (CC) (4.0 × 50 cm) eluted with MeOH to obtain two pooled fractions (Fr-1 and Fr-2). Fr-2 (4.01 g) was subjected to silica gel CC (4.0 × 15 cm, MeOH/ CH_2Cl_2, step gradient elution from 2:98 to 10:90) to obtain eight fractions, Fr-2-1 – Fr-2-8. Fr-2-6 (2.78 g) was further purified by silica gel CC (EtOAc/hexane, step gradient elution from 50:50 to 100:0) to obtain three fractions, Fr-2-6-1 – Fr-2-6-3. Fr-2-6-2 (2.24 g) was further purified by silica gel CC (EtOAc/CH_2Cl_2, step gradient elution from 50:50 to 100:0) to furnish **1b** (190 mg).

*Clonostachydiol (**1b**)*: colorless solid; $[\alpha]^{23}{}_D + 102$ (*c* 1.05, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.58) nm; IR (ATR) ν_{max} 3418, 1703, 1656, 1251, 1178, 1047, 982 cm^{-1}; for ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data in DMSO-d_6 and $CDCl_3$, see Table 1; for ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data in acetone-d_6 and ^1H NMR (500 MHz) spectroscopic data in CD_3OD, see Supplementary Information (Supplementary Table S1); HRMS (ESI-TOF) *m/z* 307.1153 $[M + Na]^+$ (calculated for $C_{14}H_{20}O_6Na$, 307.1152).

Regioselective hydrolysis of 3

Cordybislactone (**3**, 7.1 mg; isolated from *Cordyceps* sp. BCC 49294) was treated with 0.4 M aqueous K_2CO_3 (0.1 ml) in 1,4-dioxane (0.1 ml) at room temperature for 48 h. The mixture was quenched with 1 M aqueous HCl, and the resulting mixture was concentrated under reduced pressure. The residue was extracted with CH_2Cl_2 and filtered. The filtrate was concentrated in vacuo to obtain a colorless gum (8.2 mg). ^1H NMR spectrum of the crude product indicated that its major component was **4**.

Preparation of the bis-MTPA esters of 3

Cordybislactone (**3**, 2.2 mg; isolated from *Cordyceps* sp. BCC 49294) was treated with (−)-(*R*)-MTPA-Cl (14 µl) and 4-dimethylaminopyridine (DMAP, 10 mg) in CH_2Cl_2 (0.2 ml) at room temperature for 4 h. The mixture was diluted with EtOAc and washed with H_2O and 1 M $NaHCO_3$, and the organic layer was concentrated in vacuo. The residue was purified by silica gel CC (EtOAc/hexane, 20:80) to afford a bis-(*S*)-MTPA ester **5a** (4.4 mg). Similarly, bis-(*R*)-MTPA ester **5b** (3.3 mg) was prepared from **3** (2.8 mg) and (+)-(*S*)-MTPA-Cl.

Bis-(S)-MTPA ester **5a**: white foam; ^1H NMR (400 MHz, acetone-d_6) δ 7.60-7.42 (10 H, m, phenyl of MTPA × 2), 6.88 (1 H, dd, $J = 15.8$, 3.6 Hz, H-9), 6.73 (1 H, dd,

$J = 15.7, 7.2$ Hz, H-3), 6.15 (1 H, d, $J = 15.7$ Hz, H-2), 5.87 (1 H, dd, $J = 15.8, 1.7$ Hz, H-8), 5.74 (1 H, m, H-10), 5.64 (1 H, dd, $J = 7.2, 6.6$ Hz, H-4), 5.23 (1 H, dq, $J = 6.6, 6.6$ Hz, H-5), 5.02 (1 H, m, H-13), 3.57 (3 H, s, OCH_3 of MTPA), 3.52 (3 H, s, OCH_3 of MTPA), 2.12 (1 H, m, H_a-11), 1.69 (1 H, m, H_b-11), 1.62 (1 H, m, H_a-12), 1.58 (1 H, m, H_b-12), 1.39 (3 H, d, $J = 6.6$ Hz, H-6), 1.18 (3 H, d, $J = 6.4$ Hz, H-14); HRMS (ESI-TOF) *m/z* 739.1949 $[M + Na]^+$ (calculated for $C_{34}H_{34}O_{10}F_6Na$, 739.1948).

Bis-(R)-MTPA ester **5b**: white foam; ^{1}H NMR (400 MHz, acetone-d_6) δ 7.60-7.40 (10 H, m, phenyl of MTPA × 2), 6.79 (1 H, dd, $J = 15.8, 3.3$ Hz, H-9), 6.71 (1 H, dd, $J = 16.0, 6.1$ Hz, H-3), 5.84 (1 H, d, $J = 16.0$ Hz, H-2), 5.68 (2 H, m, H-4 and H-9), 5.56 (1 H, dd, $J = 15.8, 1.8$ Hz, H-8), 5.34 (1 H, m, H-5), 5.08 (1 H, m, H-13), 3.61 (3 H, s, OCH_3 of MTPA), 3.60 (3 H, s, OCH_3 of MTPA), 2.06 (1 H, m, H_a-11), 1.88 (1 H, m, H_b-11), 1.77 (1 H, m, H_a-12), 1.70 (1 H, m, H_b-12), 1.48 (3 H, d, $J = 6.7$ Hz, H-6), 1.20 (3 H, d, $J = 6.4$ Hz, H-14); HRMS (ESI-TOF) *m/z* 739.1945 $[M + Na]^+$ (calculated for $C_{34}H_{34}O_{10}F_6Na$, 739.1948).

Preparation of the bis-MTPA esters of 1b

Using the same procedures described above, bis-(*S*)-MTPA ester **6a** (5.8 mg) was prepared from **1b** (2.7 mg; isolated from BCC 4297) and (−)-(*R*)-MTPA-Cl, and bis-(*R*)-MTPA ester **6b** (4.3 mg) was prepared from **1b** (2.0 mg) and (+)-(*S*)-MTPA-Cl.

Bis-(S)-MTPA ester **6a**: white foam; ^{1}H NMR (400 MHz, acetone-d_6) δ 7.60-7.42 (10 H, m, phenyl of MTPA × 2), 6.86 (1 H, dd, $J = 16.0, 4.7$ Hz, H-9), 6.59 (1 H, dd, $J = 15.8, 8.9$ Hz, H-3), 6.26 (1 H, d, $J = 15.8$ Hz, H-2), 5.99 (1 H, dd, $J = 16.0, 1.4$ Hz, H-8), 5.93 (1 H, m, H-10), 5.41 (1 H, t, $J = 8.9$ Hz, H-4), 5.11 (1 H, m, H-5), 5.08 (1 H, m, H-13), 3.59 (3 H, s, OCH_3 of MTPA), 3.56 (3 H, s, OCH_3 of MTPA), 2.22 (1 H, m, H_a-11), 1.91 (1 H, m, H_b-11), 1.59 (1 H, m, H_a-12), 1.38 (1 H, m, H_b-12), 1.26 (3 H, d, $J = 6.3$ Hz, H-6), 1.19 (3 H, d, $J = 6.6$ Hz, H-14); HRMS (ESI-TOF) *m/z* 739.1942 $[M + Na]^+$ (calculated for $C_{34}H_{34}O_{10}F_6Na$, 739.1948).

Bis-(R)-MTPA ester **6b**: white foam; ^{1}H NMR (400 MHz, acetone-d_6) δ 7.60-7.43 (10 H, m, phenyl of MTPA × 2), 6.81 (1 H, dd, $J = 16.0, 4.6$ Hz, H-9), 6.47 (1 H, dd, $J = 15.8, 8.4$ Hz, H-3), 6.15 (1 H, d, $J = 15.8$ Hz, H-2), 5.91 (1 H, m, H-10), 5.77 (1 H, dd, $J = 16.0, 1.5$ Hz, H-8), 5.45 (1 H, t, $J = 8.4$ Hz, H-4), 5.15 (1 H, m, H-5), 5.12 (1 H, m, H-13), 3.59 (3 H, s, OCH_3 of MTPA), 3.57 (3 H, s, OCH_3 of MTPA), 2.23 (1 H, m, H_a-11), 2.01 (1 H, m, H_b-11), 1.69 (1 H, m, H_a-12), 1.65 (1 H, m, H_b-12), 1.47 (3 H, d, $J = 6.3$ Hz, H-6), 1.23 (3 H, d, $J = 6.6$ Hz, H-14); HRMS (ESI-TOF) *m/z* 739.1941 $[M + Na]^+$ (calculated for $C_{34}H_{34}O_{10}F_6Na$, 739.1948).

Methanolysis of 3

To a stirred solution of cordybislactone (**3**, 4.0 mg, 14.1 µmol) in MeOH (0.2 ml) was added NaOMe (15 mg, 0.28 mmol) at room temperature. After stirring for 1 h, the reaction mixture was quenched with 5 M aqueous HCl and extracted with EtOAc four times. The combined organic extracts were dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel CC (EtOAc/CH_2Cl_2, 50:50) to afford **7** (1.2 mg, 7.5 µmol, 53%) and **8** (1.3 mg, 6.9 µmol, 49%). Repetition of this reaction afforded additional samples of **7** (3.6 mg) and **8** (2.2 mg) for further derivatizations.

Methyl (E,4 R,5 S)-4,5-dihydroxy-2-hexenoate (7): colorless oil; $[\alpha]^{24}_D + 20$ (*c* 0.18, $CHCl_3$); IR (ATR) ν_{max} 3416, 2921, 2852, 1709, 1658, 1440, 1280, 1176, 1079, 986 cm^{-1}; ^{1}H NMR (400 MHz, acetone-d_6) δ 7.07 (1 H, dd, $J = 15.7, 4.5$ Hz, H-3), 6.08 (1 H, dd, $J = 15.7, 1.6$ Hz, H-2), 4.24 (1 H, d, $J = 5.0$ Hz, 4-O*H*), 4.14 (1 H, m, H-4), 3.84 (1 H, d, $J = 5.0$ Hz, 5-O*H*), 3.74 (1 H, m, H-5), 3.68 (3 H, s, CO_2CH_3), 1.14 (3 H, d, $J = 6.3$ Hz, H-6); ^{13}C NMR (125 MHz, acetone-d_6) δ 167.1, 149.6, 121.1, 75.7, 70.7, 51.5, 19.1; HRMS (ESI-TOF) *m/z* 183.0623 $[M + Na]^+$ (calculated for $C_7H_{12}O_4Na$, 183.0628).

Methyl (E,4 S,7 R)-4,7-dihydroxy-2-octenoate (8): colorless oil; $[\alpha]^{24}_D$ −3 (*c* 0.11, $CHCl_3$); IR (ATR) ν_{max} 2921, 2852, 1709, 1278, 1171 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 6.95 (1 H, dd, $J = 15.6, 4.5$ Hz, H-3), 6.03 (1 H, dd, $J = 15.6, 1.2$ Hz, H-2), 4.42 (1 H, d, $J = 4.6$ Hz, 4-O*H*), 4.31 (1 H, m, H-4), 3.75 (1 H, m, H-7), 3.68 (3 H, s, CO_2CH_3), 1.70-1.60 (2 H, m, H-5), 1.55-1.50 (2 H, m, H-6), 1.13 (3 H, d, $J = 6.1$ Hz, H-8); ^{13}C NMR (125 MHz, acetone-d_6) δ 167.3, 152.7, 119.7, 70.9, 67.5, 51.5, 35.8, 34.0, 24.1; HRMS (ESI-TOF) *m/z* 211.0939 $[M + Na]^+$ (calculated for $C_9H_{16}O_4Na$, 211.0941).

Methanolysis of 1b

To a stirred solution of clonostachydiol (**1b**, 11.5 mg, 40.4 µmol) in MeOH (0.3 ml) was added NaOMe (44 mg, 0.81 mmol) at room temperature. After stirring for 45 min, the reaction mixture was quenched with 5 M aqueous HCl and extracted with EtOAc four times. The combined organic extracts were dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel CC (MeOH/CH_2Cl_2, 5:95) and then silica gel CC (EtOAc/CH_2Cl_2, 50:50) to afford **7** (2.5 mg, 15.6 µmol, 39%) and **9** (2,5 mg, 13.3 µmol, 33%).

Methyl (E,4 S,7 S)-4,7-dihydroxy-2-octenoate (9): colorless oil; $[\alpha]^{23}_D + 13$ (*c* 0.17, $CHCl_3$); IR (ATR) ν_{max} 2923, 2853, 1708, 1462, 1278 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 6.95 (1 H, dd, $J = 15.6, 4.5$ Hz, H-3), 6.03 (1 H, dd, $J = 15.6, 1.0$ Hz, H-2), 4.35 (1 H, d, $J = 4.6$ Hz,

4-O*H*), 4.31 (1 H, m, H-4), 3.74 (1 H, m, H-7), 3.68 (3 H, s, CO_2CH_3), 3.64 (1 H, d, $J = 4.3$ Hz, 7-O*H*), 1.75 (1 H, m, H_a-5), 1.59-1.53 (2 H, m, H_b-5 and H_a-6), 1.48 (1 H, m, H_b-6), 1.12 (3 H, d, $J = 6.2$ Hz, H_3-8); ^{13}C NMR (125 MHz, acetone-d_6) δ 167.2, 152.7, 119.7, 71.0, 67.6, 51.5, 35.7, 33.9, 24.0; HRMS (ESI-TOF) *m/z* 211.0948 $[M + Na]^+$ (calculated for $C_9H_{16}O_4Na$, 211.0941).

Synthesis of the acetonide derivative 10

To a stirred solution of cordybislactone (**3**, 16.5 mg, 58.0 µmol) in MeOH (0.3 ml) was added K_2CO_3 (8.0 mg, 58.0 µmol) at room temperature. After stirring for 10 min, the reaction mixture was quenched with 1 M aqueous HCl and extracted with EtOAc four times. The combined organic extracts were dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to afford a crude triol. To a stirred solution of the crude triol in 2,2-dimethoxypropane (0.5 ml) was added *p*-TsOH·H_2O (ca. 5 mg, 26 µmol) at room temperature. After stirring for 5 h, the reaction mixture was quenched with 1 M aqueous $NaHCO_3$ and extracted with EtOAc three times. The combined organic extracts were dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel CC (EtOAc/CH_2Cl_2, 20:80) to afford the acetonide derivative **10** (14.8 mg, 41.5 µmol, 72% over 2 steps).

Acetonide derivative **10**: colorless gum; IR (ATR) ν_{max} 2925, 1716, 1659, 1258, 1170, 987 cm^{-1}; ^{1}H NMR (400 MHz, acetone-d_6) δ 6.93 (1 H, dd, $J = 15.6$, 4.4 Hz, H-9), 6.83 (1 H, dd, $J = 15.6$, 5.8 Hz, H-3), 6.05 (1 H, d, $J = 15.6$ Hz, H-2), 6.03 (1 H, d, $J = 15.6$ Hz, H-8), 4.99 (1 H, m, H-13), 4.74 (1 H, t, $J = 6.2$ Hz, H-4), 4.46 (1 H, dq, $J = 6.2$, 6.2 Hz, H-5), 4.34 (1 H, m, H-10), 4.24 (1 H, d, $J = 4.0$ Hz, 10-O*H*), 3.68 (3 H, s, CO_2CH_3), 1.80-1.56 (4 H, m, H-11 and H-12), 1.44 (3 H, s, H-3′), 1.32 (3 H, s, H-1′), 1.24 (3 H, d, $J = 6.2$ Hz, H-14), 1.09 (3 H, d, $J = 6.3$ Hz, H-6); ^{13}C NMR (100 MHz, acetone-d_6) δ 167.3, 166.0, 152.1, 144.9, 124.0, 120.5, 109.3, 78.5, 75.0, 71.5, 70.8, 51.5, 33.4, 32.6, 28.5, 25.8, 20.3, 16.7; HRMS (ESI-TOF) *m/z* 379.1715 $[M + Na]^+$ (calculated for $C_{18}H_{28}O_7Na$, 379.1727).

Preparation of bis-MTPA esters of 8 and 9

Using the similar procedures as described above, bis-(*S*)-MTPA ester **11a** was synthesized from diol **8** and (−)-(*R*)-MTPA-Cl, and bis-(*R*)-MTPA ester **11b** was synthesized from **8** and (+)-(*S*)-MTPA-Cl. Similarly, bis-(*S*)-MTPA ester **12a** was synthesized from **9** and (−)-(*R*)-MTPA-Cl, and bis-(*R*)-MTPA ester **12b** was synthesized from **9** and (+)-(*S*)-MTPA-Cl. NMR analyses were performed using the crude reaction products. Assignments of protons of these bis-MTPA ester derivatives were established on the basis of COSY data.

Bis-(S)-MTPA ester **11a**: ^{1}H NMR (400 MHz, acetone-d_6) δ 7.58-7.41 (10 H, m, phenyl of MTPA × 2), 6.80 (1 H, dd, $J = 15.8$, 6.8 Hz, H-3), 6.02 (1 H, d, $J = 15.8$ Hz, H-2), 5.66 (1 H, m, H-4), 5.11 (1 H, m, H-7), 3.73 (3 H, s, CO_2CH_3), 3.60 (3 H, s, OCH_3 of MTPA), 3.54 (3 H, s, OCH_3 of MTPA), 1.67 (1 H, m, H_a-5), 1.63 (1 H, m, H_b-5), 1.53 (1 H, m, H_a-6), 1.51 (1 H, m, H_b-6), 1.29 (3 H, m, H_3-8).

Bis-(R)-MTPA ester **11b**: ^{1}H NMR (400 MHz, acetone-d_6) δ 7.58-7.41 (10 H, m, phenyl of MTPA × 2), 6.82 (1 H, dd, $J = 15.8$, 5.6 Hz, H-3), 5.87 (1 H, d, $J = 15.8$ Hz, H-2), 5.75 (1 H, m, H-4), 5.20 (1 H, m, H-7), 3.69 (3 H, s, CO_2CH_3), 3.57 (3 H, s, OCH_3 of MTPA), 3.53 (3 H, s, OCH_3 of MTPA), 1.96 (1 H, m, H_a-5), 1.95 (1 H, m, H_b-5), 1.82 (1 H, m, H_a-6), 1.78 (1 H, m, H_b-6), 1.28 (3 H, d, $J = 6.4$ Hz, H-8).

Bis-(S)-MTPA ester **12a**: ^{1}H NMR (400 MHz, acetone-d_6) δ 7.55-7.43 (10 H, m, phenyl of MTPA × 2), 6.92 (1 H, dd, $J = 15.8$, 5.7 Hz, H-3), 6.08 (1 H, dd, $J = 15.8$, 1.4 Hz, H-2), 5.76 (1 H, m, H-4), 5.12 (1 H, m, H-7), 3.72 (3 H, s, CO_2CH_3), 3.57 (3 H, br s, OCH_3 of MTPA), 3.53 (3 H, br s, OCH_3 of MTPA), 1.96 (1 H, m, H_a-5), 1.87 (1 H, m, H_b-5), 1.58 (1 H, m, H_a-6), 1.57 (1 H, m, H_b-6), 1.18 (3 H, d, $J = 6.2$ Hz, H-8).

Bis-(R)-MTPA ester **12b**: ^{1}H NMR (400 MHz, acetone-d_6) δ 7.56-7.41 (10 H, m, phenyl of MTPA × 2), 6.71 (1 H, dd, $J = 15.8$, 5.5 Hz, H-3), 5.78 (1 H, dd, $J = 15.8$, 1.4 Hz, H-2), 5.66 (1 H, m, H-4), 5.19 (1 H, m, H-7), 3.70 (3 H, s, CO_2CH_3), 3.57 (3 H, br s, OCH_3 of MTPA), 3.54 (3 H, br s, OCH_3 of MTPA), 1.72 (2 H, m, H-5), 1.70 (1 H, m, H_a-6), 1.68 (1 H, m, H_b-6), 1.37 (3 H, d, $J = 6.2$ Hz, H-8).

Biological assays

Cytotoxic activities against the tumor cell-lines, NCI-H187 (human small-cell lung cancer), MCF-7 (human breast cancer), and KB (oral human epidermoid carcinoma), were evaluated using the resazurin microplate assay [12]. Antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra was evaluated using the green fluorescent protein (GFP)-based microplate assay [13]. The MIC values of the standard antituberculosis drugs (positive control), isoniazid and rifampicin, were 0.0469 and 0.0125 µg/ml, respectively.

Acknowledgements Financial support from the Thailand Research Fund (Grant No. DBG5980002) and JSPS KAKENHI (Grant Numbers 16H01127, 16H00999, and a Grant-in-aid for Scientific Research (A) 26253001) is gratefully acknowledged. Ojima thanks JSPS KAKENHI, Grant-in-aid for Scientific Research Priority Area: Middle Molecular Strategy. We are grateful to Mr Prasert Srikitikulchai for identification of the fungus *Xylaria* sp. BCC 4297.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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