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Tropolactones A–D, four meroterpenoids from a marine-derived fungus of the genus *Aspergillus*

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We dedicate this paper to Professor Rodney Croteau on occasion of his 60th birthday.

Abstract

Four cytotoxic meroterpenoids, tropolactones A–D, were isolated from the whole broth extract of a marine-derived fungus of the genus *Aspergillus*. The structures of the meroterpenoids were established through a variety of two-dimensional NMR techniques. The absolute configuration of tropolactone A was determined using the modified Mosher method. Tropolactones A–C contain an interesting substituted 2,4,6-cycloheptatriene (tropone) ring, which presumably arises through an oxidative ring expansion from tropolactone D. Tropolactones A, B and C showed in vitro cytotoxicity against human colon carcinoma (HCT-116) with IC_{50} values of 13.2, 10.9 and 13.9 μg/mL.

1. Introduction

Meroterpenoids, compounds of mixed terpene and polyketide biosynthesis, have been isolated from plants, marine invertebrates and microorganisms. In the 1970s, the meroterpenes andibenin B **(1)** (Dunn et al., 1976) and andilesin A (**2**) (Dunn et al., 1978) were isolated from the terrestrial fungus *Aspergillus variecolor*. Incorporation experiments with labeled acetate revealed the andibenin class of natural products were derived from a sesquiterpene and a tetraketide precursor, with additional *C*-methyl groups being introduced by S-adenosyl methionine (Bartlett et al., 1981). Further elaboration of this class of compounds through a variety of oxidative processes gives rise to broad structural diversity.

In our continued investigation of marine-derived fungi as a source of bioactive metabolites (see Bugni and Ireland, 2004 for a review; Tan et al., 2004; Rowley et al., 2003; Cueto et al., 2002) we have identified tropolactones A–D (**3**–**6**), four new cytotoxic meroterpenoids from a marine-derived *Aspergillus* sp. (strain CNK-371), which was isolated from an unidentified sponge collected at Manele Bay, Hawaii. The four new compounds are of a similar structural class to andilesin A (**2**). Interestingly, tropolactones A–C (**3**–**5**) contain a substituted 2,4,6-cycloheptatriene-2-one (tropone) ring, which presumably arises from an oxidative ring expansion of the 2,5-cyclohexadiene-2-one ring of tropolactone D (**6**).

2. Results and discussion

Fungal strain CNK-371 was cultured (20 × 1 L) in a seawater-based marine nutrient medium. The whole culture (broth and mycelium) was extracted with ethyl acetate and the combined crude extracts were sequentially purified by flash C-18 chromatography, Sephadex LH-20 chromatography and normal phase HPLC to yield tropolactones A–D (**3**–**6**) in 8.4, 5.3, 17.1, and 29.8 mg yields, respectively (0.4 mg to 1.5 mg/L).

Tropolactone A (**3**) was isolated as a colorless oil ($[\alpha]_D$ −48.0° (*c* 1.8, CH_2Cl_2)). The molecular formula of **3** was determined to be $C_{26}H_{34}O_7$ based on HRMALDIMS ($[M + Na]^+$ $m/z = 481.2217$). The ^{13}C NMR spectrum of **3** revealed three carbonyl carbons (δ_C 181.8, 170.9 and

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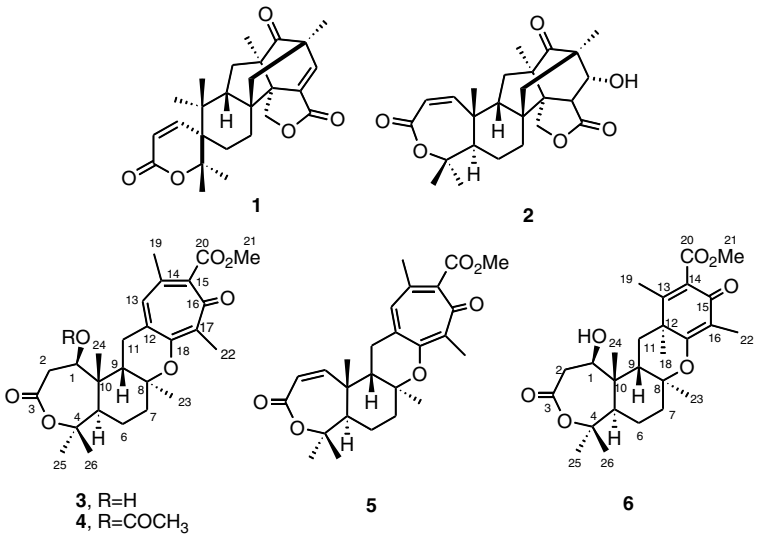


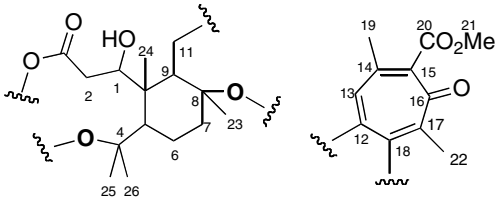
168.8 ppm) as well as six olefinic carbons. The ^{1}H NMR spectrum revealed the presence of seven methyl singlets, one of which was attached to oxygen, while the DEPT experiment showed three sp^{3} quaternary carbons (δ_C 84.9, 80.2 and 40.3 ppm). The NMR data accounted for six of the ten double bond equivalents, leaving a structure that contained four rings.

Two partial structures for **3** were determined by analyses of ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC experiments. Partial structure **a** was defined on the basis of COSY correlations from the heteroatom substituted methine (C1) at δ_H 4.28 (*dd*, $J = 4.5$, 3.0) to the C2 methylene protons at δ_H 3.20 (*dd*, $J = 14.6$, 4.5 Hz) and δ_H 2.48 (*dd*, $J = 14.6$, 3.0 Hz). The lactone carbonyl at C3 was established based on HMBC correlations from H1, H2a and H2b to an ester carbonyl carbon at δ_C 170.9. The C-24 bridgehead methyl group (δ_H 1.25, *s*) was established by HMBC correlations to C1 (δ_C 69.5) and C5 (δ_C 48.3) and from the H5 methine (δ_H 2.15, *m*) to the C10 quaternary carbon (δ_C 43.0). The C25 and C26 methyl singlets were determined to be geminal based on mutual HMBC correlations to each other and correlations from methyl protons (δ_H 1.35 and 1.47) to the oxygen substituted C4 quaternary carbon (δ_C 84.9), and to C5 (δ 48.3).

The tetrasubstituted B-ring of **3** was assigned on the basis of a series of HMBC and COSY NMR correlations, with the key correlations from H5 to C6 (δ_C 22.9) and C7 (δ_C 39.9) and the enantiotopic methylene proton H7 α (δ_H 2.18, *m*) to the quaternary carbon at C8 (δ_C 80.2) and the methine carbon at C9 (δ_C 46.9). Partial structure **a** was completed with a second bridgehead methyl group assigned at C8 on the basis of observed HMBC correlations from the protons of the C23 methyl singlet (δ_C 1.38) to C7, C8 and C9.

The tropone ring of partial structure **b** was assigned by analysis of HMBC data, utilizing correlations from H13 (δ_H 6.87, *s*) and the protons of the C19 and C22 methyl groups (δ_H 2.12 and 2.05, respectively). Briefly, H13 correlated to the quaternary carbons C18 (δ_C 159.0), C15 (δ_C 142.9) and the C19 methyl (δ_C 23.7). The C19 methyl singlet showed HMBC correlations to C13 (δ_C 135.4), C14 (δ_C 138.3) and C15, while the C22 methyl singlet showed correlations to carbonyl carbons C16 (δ_C 181.8), C17 (δ_C 135.1) and C18 (δ_C 159.0). These HMBC correlations,overall, revealed a pentasubstituted 2,4,6 cycloheptatriene-1-one (tropone) ring. Tropone and tropolone containing secondary metabolites are uncommon, but have been encountered as fungal metabolites such as malettinin A (Angawi et al., 2003), stipitatic acid (Dewar, 1944) and epolone (Cai et al., 1998). Based on ^{1}H and ^{13}C NMR chemical shifts of C21, and a HMBC correlation from the protons of methyl singlet C21 (δ_H 3.74) to C20 (δ_C 168.9), the two remaining carbons were determined to be a carbomethoxy fragment. A weak 4-bond HMBC correlation from H19 to C20, allowed the carbomethoxy group to be placed at C15.

Partial structures **a** and **b** were combined based on HMBC correlations and chemical shift data. A C11–C12 connection was assigned based on a HMBC correlation from H13 to C11, while the pyran ring closure between C8 and C18 was assigned on the basis of the downfield chemical shifts of the two carbons (δ_C 80.2 and 159.0, respectively). To account for the remaining degree of unsaturation, the C3 carbonyl and the oxygen substituted C4 quaternary carbon (δ_C 84.9) was cyclized to form a lactone, thus completing the planar structure of **3** as a tropone-containing meroterpenoid.



The relative stereochemistry of **3** was determined by a 2D NOESY experiment (Fig. 1a). The C5/C10 *trans* fused ring junction was established by NOE correlations between H24 and H9 and between H24 and H26 establishing one face of the molecule, while correlations between H5 and H25 established the *trans* junction. The pseudo axial C23 methyl group was established to be on the same face as H1 based on NOE correlations between H1 and H23 and between H5 and H23. The NOE interaction between H1 and H23 was initially a surprise until modeling revealed a 2.2 Å interatomic distance between the protons when the B ring adopts a pseudo-boat configuration. The NOE correlation data also showed that the C8/C9 ring junction was *trans*, which allowed for full assignment of the relative configuration of **1**.

Modified Mosher's analysis was used to assign the absolute configuration of tropolactone A (**3**) (Dale et al., 1969; Ohtani et al., 1991). Briefly, **3** was converted to the (*R*)- and (*S*)-MTPA esters by reaction with (*S*) and (*R*)-MTPA-Cl in CH_2Cl_2 using catalytic DMAP. Analysis of the proton chemical shift $\Delta\delta$ values of the (S)- and (*R*)-MTPA esters ($\Delta\delta = \delta S - \delta R$) show that C1 has the *R* configuration (Fig. 1b). Mosher's analysis coupled with the establishment of the relative configuration by NOE correlations gave the absolute configuration of **3** as 1*R*, 5*R*, 8*S*, 9*S*, 10*S*.

Tropolactone B (**4**) was isolated as a colorless oil ($[\alpha]_D$ −30.0° (*c* 0.3, CH_2Cl_2)). The molecular formula of **4** was determined to be $C_{28}H_{36}O_8$ based on HRMALDI MS ($[M + Na]^+$ $m/z = 523.2289$), a difference of $C_2H_2O_2$ from **3**. The ^1H and ^{13}C NMR spectra of **4** were nearly identical to **3**, with the most significant difference being the additional ^{13}C resonances at δ_C 21.2 and 170.3 ppm (see Table 1) and a downfield shift of H1 at δ_H 4.28 ppm to δ_H 5.27 ppm. The combination of data allowed for the assignment of **4** as the C1 *O*-acetyl derivative of **3**. Analysis of the 2D NOESY showed that **4** has the same relative configuration as **3**. It can be assumed that the absolute stereochemistry of **4** at C1 was the same as in **3** based on the similar optical rotation values. Therefore the absolute stereochemistry of **4** was assigned as 1*R*, 5*R*, 8*S*, 9*S*, 10*S*.

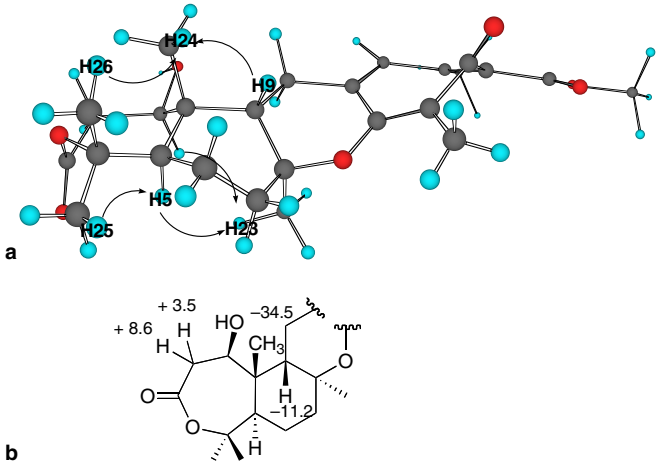


Fig. 1. Relative and absolute stereochemistry of tropolactone A (**3**): (a) Arrows indicate NOE correlations used to establish the relative configuration; (b) $\Delta\delta_{S\text{-}R}$ values (Hz) for the (*R*)- and (*S*)-MTPA esters of **3**.

Further analysis of the crude extract of CNK-371 revealed tropolactone C (**5**), which was also isolated as a clear oil ($[\alpha]_D$ −78.0° (*c* 1.1, CH_2Cl_2)). The molecular formula was determined to be $C_{26}H_{32}O_6$ by HRMALDI MS ($[M + Na]^+$ $m/z = 441.2271$). The major differences between tropolactone C (**5**) and tropolactone A (**3**) were the loss of the ^1H NMR signals at δ_H 4.28, 3.20, 2.48 ppm and the appearance of two olefinic signals at δ_H 7.19 (*d*, $J = 11.4$ Hz) and 5.95 (*d*, $J = 11.4$ Hz). This allowed the structure of **5** to be assigned as the 1,2-dehydro derivative of **3**. Analysis of the 2D NOESY data for **5** gave the relative configuration being consistent with **3** and allowed for the assignment of the absolute stereochemistry of **5** as 5*R*, 8*S*, 9*S*, 10*S*.

Tropolactone D (**6**), which was the major secondary metabolite in the culture, was isolated as a colorless oil ($[\alpha]_D$ −179° (*c* 0.5, CH_2Cl_2)). The molecular formula of **6** was determined to be $C_{26}H_{36}O_7$ by HRMALDI MS ($[M + Na]^+$ $m/z = 483.2333$), a molecular weight difference of two from tropolactone A (**3**). The ^1H and ^{13}C NMR spectroscopic data for **6** showed a number of similarities to **4**, but overall there were significant differences. The ^1H NMR spectrum of **6** revealed the presence of eight methyl singlets, while the ^{13}C NMR spectrum showed only four olefinic carbons and an additional sp^3 quaternary carbon. The C1–C10 (A ring) lactone segment of **6** appeared to be intact, while the tropone ring was no longer present. The HMBC NMR spectroscopic data showed a correlation from the C18 methyl protons (δ_H 1.45 *s*) to C12 (δ_C 45.6), C13 (δ_C 157.1) and C17 (δ_C 173.2) thus establishing **6** as the ring contracted 2,5 cyclohexadiene-1-one derivative of **3**. The relative configuration of the C18 methyl group was determined to be *syn* to the C23 methyl group on the basis of 2D NOESY NMR correlation, while the remaining centers were consistent with tropolactones A–C (**3**–**5**). The absolute stereochemistry was thus assigned as 1*R*, 5*R*, 8*S*, 9*S*, 10*S*, 12*S*.

Tropolactones A–C (**3**–**5**) showed weak cytotoxicity against human colon adenocarcinoma cells (HCT-116) with IC_{50} values of 13.2, 10.9 and 13.9 μM, respectively.

2.1. Concluding remarks

The biosynthesis of meroterpenoids of similar structure to the tropolactones proceeds through an alkylation of methyl 2,4-dihydroxy-3,5,6-trimethylbenzoate (**7**) with farnesyl pyrophosphate (**8**) to give cyclohexadienone **9**. In the case of andibenin B (**1**), intermediate **9** undergoes an epoxide induced cyclization cascade to give triene (**10**), followed by a [4 + 2] cycloaddition to give the required carbon skeleton for **1** (Simpson et al., 1997). We hypothesize the carbon backbone in the tropolactones derives from the same triene intermediate **10**, which undergoes enol tautomerization to give enol **11**. Subsequent

Table 1
NMR spectroscopic data for tropolactone C (**5**) and tropolactone D (**6**) in acetone-d_6

#	Tropolactone A (**3**)			Tropolactone B (**4**)	
	^{1}H (mult, *J* (Hz))	^{13}C	HMBC correlations	^{1}H (mult, *J* (Hz))	^{13}C
1	4.28 (*dd*, 4.5, 30)	69.5	C-3, C-9	5.27 (*dd*, 4.5, 3.0)	71.2
2α	3.20 (*dd*, 14.6, 4.5)	42.9	C-l, C-3	3.34 (*dd*, 15.0, 4.5)	38.8
2β	2.48 (*dd*, 14,6, 3.0)		C-1, C-3, C-10	2.54 (*dd*, 15.0, 3.0)	
3	–	170.9		–	I69.8
4	–	84.9		–	85.l
5	2.15 (*m*)	48.3	C-1, C4, C-6, C-7, C10, C-24, C-25, C-26	2.11 (*m*)	47.5
6α	1.50 (*m*)	22.9	C-7, C-8	1.62 (*m*)	22.4
6β	1.44 (*m*)			1.40 (*m*)	
7α	2.18 (*m*)	39.9	C-8	2.30 (*m*)	39.6
7β	1.86 (*m*)			1.60 (*m*)	
8	–	80.2		–	79.6
9	1.95 (*m*)	46.9	C-l, C8, C-10, C-II, C-24	2.04 (*dd*, 4.5, 3.2)	46.3
10	–	43.0		–	41.7
11α	2.44 (*m*)	30.9		2.78 (*dd*, 14.5, 3.2)	29.7
11β	3.07 (*dd*, 14,5, 4.5)		C-9, C-12, C-13, C-18	3.00 (*dd*, 14.5, 4.5)	
12	–	137.9		–	136.5
13	6.87 (*s*)	135.4	C-11, C-15, C-18, C-19	6.92 (*s*)	135.6
14	–	138.3		–	138.0
15	–	142.9		–	142.9
16	–	181.8		–	181.5
17	–	135.1		–	134.9
18	–	159.0		–	158.5
19	2.12 (*s*)	23.7	C-13, C-14, C-15	2.02 (*s*)	23.5
20	–	168.9		–	168.7
21	3.74 (*s*)	52.0	C-20	3.78 (*s*)	51.9
22	2.05 (*s*)	14.8	C-16, C-17, C-18	2.09 (*s*)	14.6
23	1.38 (*s*)	26.4	C-7, C-S, C-9	1.47 (*s*)	25.9
24	1.25 (*s*)	16.8	C-l, C-5, C-9, C-10	1.42 (*s*)	17.4
25	1.35 (*s*)	26.7	C-4, C-5, C-26	1.44 (*s*)	26.5
26	1.47 (*s*)	30.5	C-4, C-5, C-25	1.56 (*s*)	30.3
1C=O	–	–		–	170.3
ICH3	–	–		2.16 (*m*)	21.2

stereospecific attack of the C17 phenol oxygen to the C9 olefin produces the pyran ring structure **12** of the tropolactones (Fig. 2) (Dillen et al., 1989). It is possible however that **9** proceeds to the tetracyclic structure **12** directly through an epoxide induced cyclization. Oxidation of the C2 alcohol followed by an enzymatic Baeyer–Villager oxidation would yield the seven membered ring lactone **13** (DeJesus et al., 1987).

The most intriguing aspect of the biosynthesis of the tropolactones is the ring expansion from the 2,5-cyclohexadiene-1-one **13** to the tropone ring of **15**. The few biosynthetic studies of tropone and tropolone containing compounds propose an oxidative ring expansion mechanism (O'Sullivan and Schwab, 1995). In the case of the tropolone containing colchicine alkaloids the mechanism involves the formation of a cyclopropyl intermediate which opens to form the tropolone. We hypothesize that the ring expansion in tropolactones A–C proceeds via a radical cyclopropanation mechanism to give stabilized radical intermediate **14**, which can then undergo ring expansion to provide tropolone **15**. Further oxidative modifications of **15** result in tropolactones A–C (**3**–**5**).

3. Experimental

3.1. General procedures

Optical rotations were measured at 25 °C on a Rudolph Research Autopol III polarimeter with a path length of 10 cm at the sodium line (589 nm). UV spectra were obtained on a Beckman Coulter DU 640. IR spectra were recorded as a thin film on a NaCl disc using a Perkin Elmer 1600 FTIR. NMR spectra were recorded on a Varian Inova spectrometer with proton data being recorded at 300 MHz and ^{13}C spectra recorded at 75 MHz, using residual solvent as an internal reference (acetone-d_6, δ_H 2.05 ppm, δ_C 30.6 ppm). High-resolution mass spectral data were measured using MALDI-FTMS.

3.2. Fungal isolation and identification

Fungal strain CNK-371 was obtained from an unidentified sponge collected at 40 feet from Manele Bay, Lanai, Hawaii in 1997. A portion of the sponge was placed into a vial containing sterilized seawater (20 mL) and crab meal

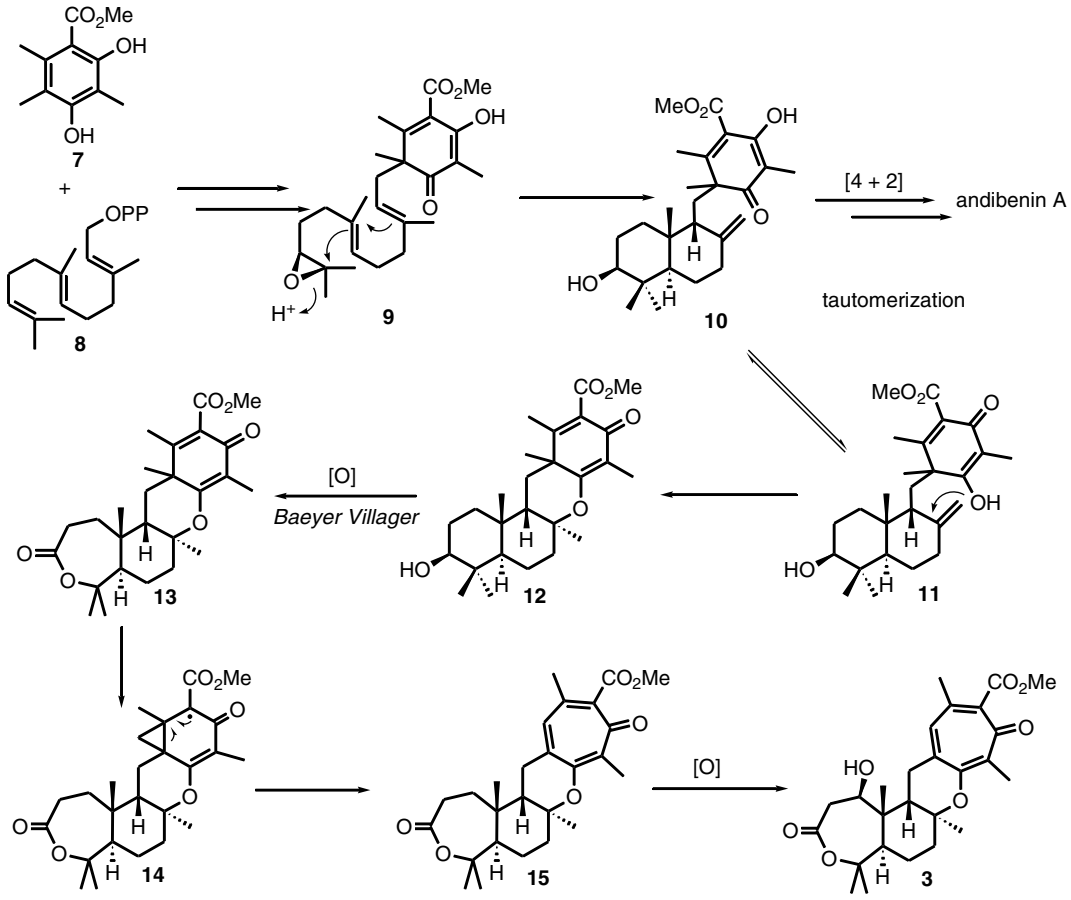


Fig. 2. Proposed biosynthesis of the tropolactones.

(2 g) and allowed to incubate at room temperature. Following incubation, 50 mL of the culture media was inoculated onto a media containing yeast extract (5 g/L), peptone (5 g/L), glucose (10 g/L), agar (16 g/L) and penicillin G/streptomycin sulfate (150 mg/L) using the spread plate technique. Fungal isolate CNK-371 was isolated from this plate and identified as *Aspergillus* sp. by Microbial ID, Inc. (Newark, DE, USA).

3.3. Cultivation of strain CNK-371

Strain CNK-371 was cultivated without shaking in 2.8 L Fernbach flasks (20×1 L) at 25 °C in a seawater-based marine nutrient medium containing mannitol (5 g/L), hydrolyzed fish solubles (2 mL/L), Menhaden meal (2 g/L) and kelp powder (2 g/L). After 30 days, the combined culture broth and fungal mass were extracted with EtOAc (25 L), dried (anhydrous Na_2SO_4), with the solvent removed in vacuo.

3.4. Compound isolation

The crude extract of strain CNK-371 was first subjected to C-18 silica flash chromatography using gradient elution (100% H_2O to 100% MeOH) followed by size exclusion chromatography (Sephadex LH-20, *iso*-octane:toluene: MeOH (3:1:1)). The final purification was performed using normal phase HPLC (Dynamax semi-preparative column, silica gel, 60 Å, 10 mm × 250 mm) with EtOAc (100%), yielding **3** (8.4 mg), **4** (5.3 mg), **5** (17.1 mg), and **6** (29.8 mg).

3.4.1. Tropolactone A (**3**)

Colorless oil, $[\alpha]_D$ −48° (*c* 1.8, CH_2Cl_2); UV λ_{max} ($CHCl_3$): 272 (ε 23,890), 264 (ε 26,112), 227 (ε 12,600) nm; IR (film, NaCl) ν_{max} 3463, 2942, 1731, 1713, 1613, 1513, 1437, 1384, 1284, 1178, 1131, 1067 cm^{-1}; $C_{26}H_{34}O_7$ by HRMALDI ($[M + Na]^+$ $m/z = 481.2217$; calc. 481.2197); For ^1H and ^{13}C NMR spectroscopic data, see Table 1.

3.4.2. Tropolactone B (**4**)

Colorless oil, $[\alpha]_D$ −30° (*c* 0.3, CH_2Cl_2); UV λ_{max} ($CHCl_3$): 271 (ε 18,760), 264 (ε 20,390), 231 (ε 8700) nm; IR (film, NaCl) ν_{max} 3436, 2954, 1731, 1619, 1531, 1437, 1378, 1231, 1178, 1125, 1067 cm^{-1}; $C_{28}H_{36}O_8$ by HRMALDI ($[M + Na]^+$ *m/z* obsd. 523.2289; calc. 523.2302); For ^1H and ^{13}C NMR spectroscopic data, see Table 1.

3.4.3. Tropolactone C (**5**)

Colorless oil, $[\alpha]_D$ −78° (*c* 1.1, CH_2Cl_2); UV λ_{max} ($CHCl_3$): 263 (ε 14,800), 229 (ε 13,330) nm; IR (film, NaCl)

Table 2
NMR spectroscopic data for tropolacetone C (**5**) and tropolactone D (**6**) in acetone-d_6

#	Tropolactone C (**5**)		Tropolactone D (**6**)	
	^{1}H (mult, *J* (Hz))	^{13}C	^{1}H (mult, *J* (Hz))	^{13}C
1	7.19 (*d*, 11.4)	156.4	4.28 (*t*, 3.9, 3.0)	69.3
2α	5.95 (*d*, 11.4)	124.3	3.15 (*dd*, 14.6, 3.9)	42.8
2β			2.45 (*dd*, 14.6, 3.0)	
3	–	167.1	–	170.7
4	–	85.1	–	84.5
5	2.28 (*m*)	54.3	2.19 (*dd*, 13. 1,2.4)	46.6
6α	1.60 (*m*)	22.4	1.75 (*m*)	22.8
6β	1.95 (*m*)		1.50 (*m*)	
7α	2.30 (*m*)	39.7	2.10 (*m*)	41.3
7β	2.18 (*m*)		2.10 (*m*)	
8	–	S0.5	–	86.2
9	2.20 (*dd*, 42, 3.4)	45.8	1.40 (*m*)	49.4
10	–	42.4	–	43.4
11α	2.30 (*m*)	30.2	1.90 (*m*)	30.7
11β	2.96 (*dd*, 16.0, 4.2)		2.51 (*dd*, 15.5, 3.9)	
12	–	137.6	–	45.6
13	6.97 (*s*)	134.7	–	157.1
14	–	138.1	–	132.9
15	–	142.8	–	182.9
16	–	181.6	–	118.6
17	–	134.9	–	173.2
18	–	158.5	1.45 (*s*)	32.8
19	2.17 (*s*)	23.5	1.97 (*s*)	15.9
20	–	168.7	–	167.9
21	3.79 (*s*)	51.9	3.74 (*s*)	51.8
22	2.10 (*s*)	14.7	1.71 (*s*)	8.1
23	1.36 (*s*)	25.8	1.62 (*s*)	27.2
24	1.52 (*s*)	21.0	l.11 (*s*)	16.4
25	1.46 (*s*)	24.9	1.28 (*s*)	26.5
26	1.46 (*s*)	32.7	1.43 (*s*)	30.2

ν_{max} 3436, 2954, 1725, 1689, 1531, 1443, 1231, 1125 cm^{-1}; $C_{26}H_{32}O_6$ by HRMALDI ($[M+H]^+$ *m/z* obsd. 441.2271; calc. 441.2272); For ^{1}H and ^{13}C NMR spectroscopic data, see Table 2.

3.4.4. Tropolactone D (*6*)

Colorless oil, $[\alpha]_D$ −179° (*c* 0.5, CH_2Cl_2); UV λ_{max} ($CHCl_3$): 287 (ε 2280), 244 (ε 3530), 227 (ε 6820) nm; IR (film, NaCl) ν_{max} 3436, 2942, 1725, 1654, 1607, 1448, 1390, 1290, 1225, 1155, 1125, 1061 cm^{-1}; $C_{26}H_{36}O_7$ by HRMALDI ($[M+Na]^+$*m/z* obsd. 483.2333; calc. 483.2353); For ^{1}H and ^{13}C NMR spectroscopic data, see Table 2.

3.4.5. Preparation of Mosher ester derivatives (*3a* and *3b*) of *3*

A mixture of **3** (1.7 mg), (*S*)-MTPA-Cl (5 mg), and 4-(dimethylamino)pyridine (5.4 mg, 45 μM) in CH_2Cl_2 (0.5 mL) was stirred at room temperature for 72 h. The solvent was removed under N_2 and the residue was purified by C-18 HPLC using MeOH (100%) to give the (*R*)-MTPA ester **1a** (2.2 mg). The same experimental procedure was followed using (*R*)-MTPA-Cl for the production of the corresponding (*S*)-MTPA ester **3b** (1.9 mg).

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