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TMC-171A, B, C and TMC-154, Novel Polyketide Antibiotics Produced by *Gliocladium* sp. TC 1304 and TC 1282

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Four new antibiotics, TMC-171A (**2**), B (**3**), C (**4**) and TMC-154 (**5**) have been isolated from the fermentation of fungal strains *Gliocladium* sp. TC 1304 and TC 1282, respectively. Spectroscopic and degradation studies have shown that TMC-171s and TMC-154 were new members of the TMC-151 class of antibiotics, unique polyketides modified with a D-mannose and a D-mannitol or a D-arabitol. These compounds showed moderate cytotoxicity to various tumor cell lines.

In our continuing search for microbial metabolites, especially for the products of Hypocreales, we have reported novel cysteine proteinase inhibitors, TMC-52 A~D, and novel cytotoxic antibiotics, TMC-151 A~F, produced by *Gliocladium* spp.[1,2] Among them, TMC-151s had been isolated from the fermentation of *Gliocladium catenulatum* TC 1280, and were novel polyketides containing D-mannnopyranoside and D-mannitol or D-arabitol (Fig. 1). They were the first fungal metabolites having an erythromycin-type polyketide structure.[2] Further investigation resulted in the isolation of new members of this class of antibiotics, designated as TMC-171 A~C (**2**~**4**) and TMC-154 (**5**) from *Gliocladium* sp. TC 1304 and TC 1282, respectively, which were taxonomically closely related to *Gliocladium roseum* BAINIER. This paper describes the isolation, physico-chemical properties, structure determination and biological activity of **2**~**5**.

Results

Isolation

Isolation and purification procedures for TMC-171 A~C (**2**~**4**) and TMC-154 (**5**) are summarized in Figs. 2 and 3, respectively. TMC-171s and TMC-154 were extracted with 1-butanol from the production media (solid state) of *Gliocladium* sp. TC 1304 and TC 1282, respectively. The extracts were purified by a combination of solvent partition and successive column chromatography. All compounds were finally obtained as colorless powders.

Physico-chemical Properties

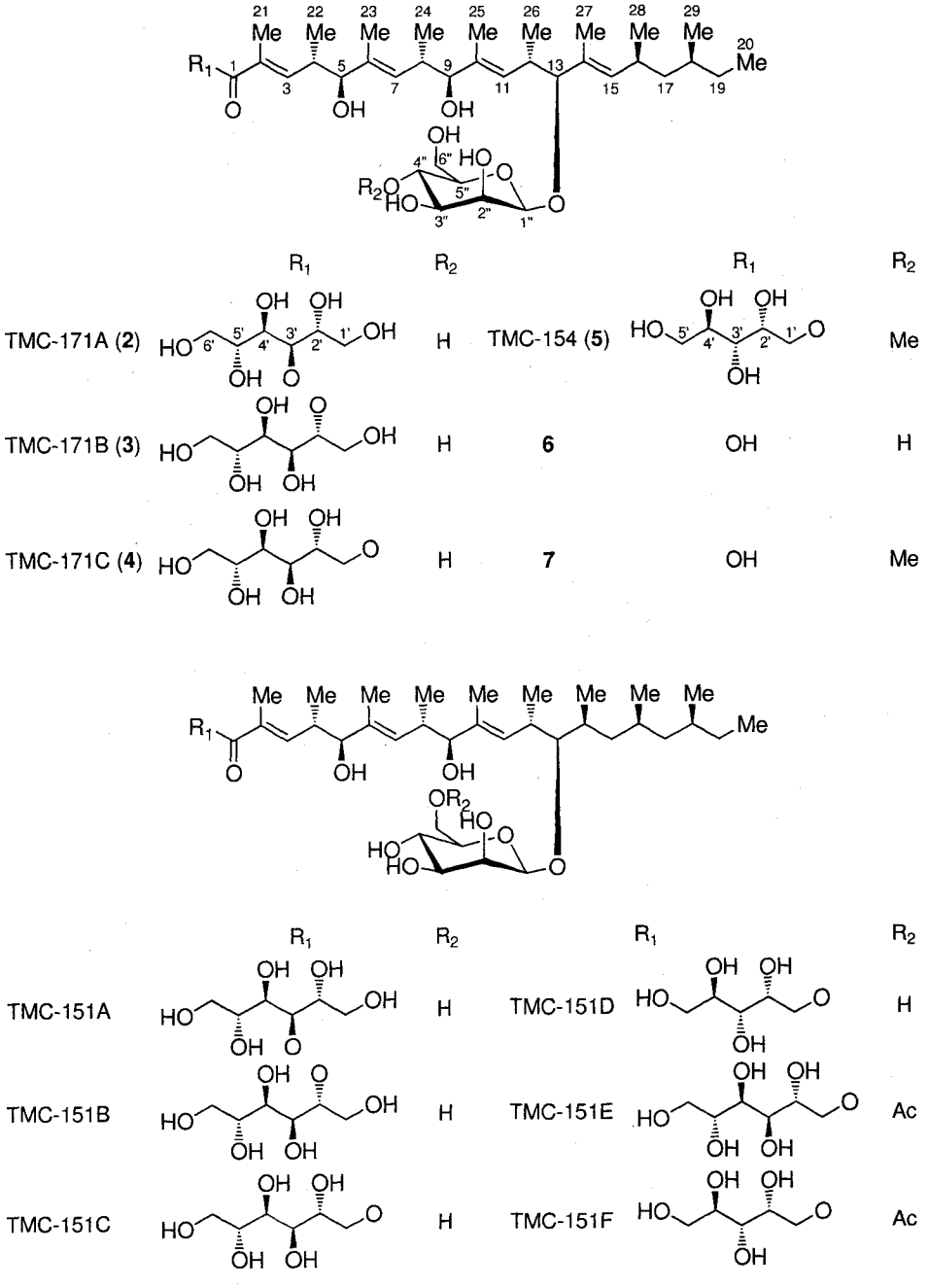
The physico-chemical properties of **2**~**5** are summarized in Table 1. They had almost identical UV and IR absorption spectra to each other. Their IR spectra showed in common the presence of alkyl groups (2950 and 2920 cm^{-1}), an ester carbonyl group (1700 cm^{-1}), and hydroxyl or ether groups (1075 and 1020 cm^{-1}). The IR spectrum of TMC-171C (**4**) is shown in Fig. 4. These data along with their producing organisms being *Gliocladium* species, suggested that TMC-171s and TMC-154 were analogs of TMC-151s.

Structure Determination

TMC-171A (**2**), B (**3**) and C (**4**)

The molecular formulas of **2**~**4** were all determined as $C_{41}H_{72}O_{15}$ on the basis of their HR-ESI-MS and ^{1}H and ^{13}C NMR data, which differed from those of TMC-151A~C by two hydrogens. The ^{13}C and ^{1}H NMR data of **2**~**4** are shown in Tables 2 and 3, respectively.

Fig. 1. Structures of TMC-171A (**2**), B (**3**), C (**4**), TMC-154 (**5**), their derivatives (**6**, **7**), and related compounds.



The ^{13}C NMR spectra of **2**~**4** displayed 41 signals composed of CH_3–×10, $-CH_2$–×2, $-CH_2$–O–×3, >CH–×5, >CH–O–×11, anomeric CH–×1, –CH=×4, >C=×4 and ester carbonyl C×1. The ^1H NMR spectra of **2**~**4** showed eleven hydroxyl protons at δ 3.84 ~ 4.84. These spectra of **2**~**4** corresponded well to those of TMC-151 A~C, respectively, except for the following signals: the alkyl chain at C-14 and C-15, the methyl group at C-27 and the anomeric position at C-1″ in the mannoside moiety. The sp^2 carbons at C-14 (δ 129.8) and C-15 (δ 137.5) for **2**~**4** were observed in place of the corresponding sp^3 carbons (δ 33.0 and 42.1) for TMC-151 A~C. The methyl carbons at C-27 (δ 11.0 ~ 11.2) and the anomeric carbons at C-1″ (δ 96.1) for **2**~**4** were shifted higher field relative to those for TMC-151 A~C (C-27: δ 15.8, C-1″: δ 101.3). From these data together with small coupling constants, $^1J_{C\text{-}1'',\ H\text{-}1''}$=

Fig. 2. Isolation and purification procedure for TMC-171A (**2**), B (**3**) and C (**4**).

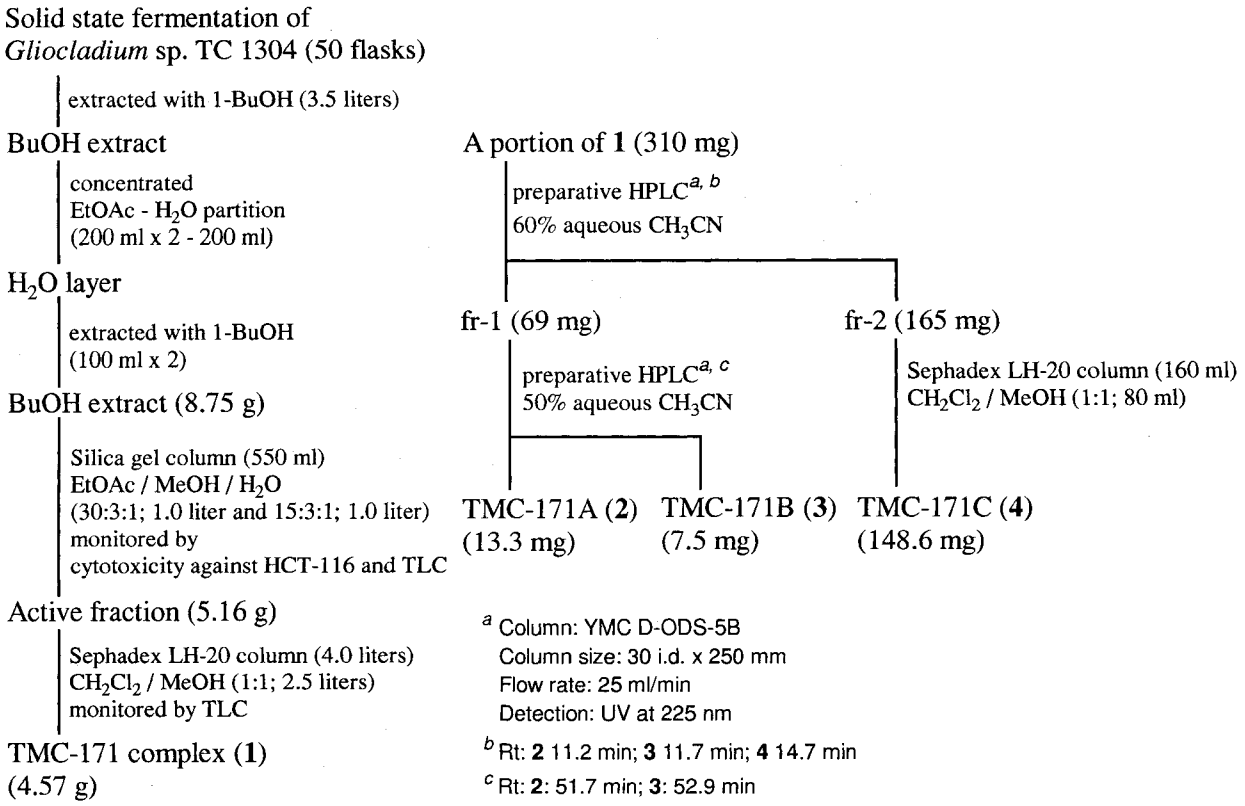


Fig. 3. Isolation and purification procedure for TMC-154 (**5**).

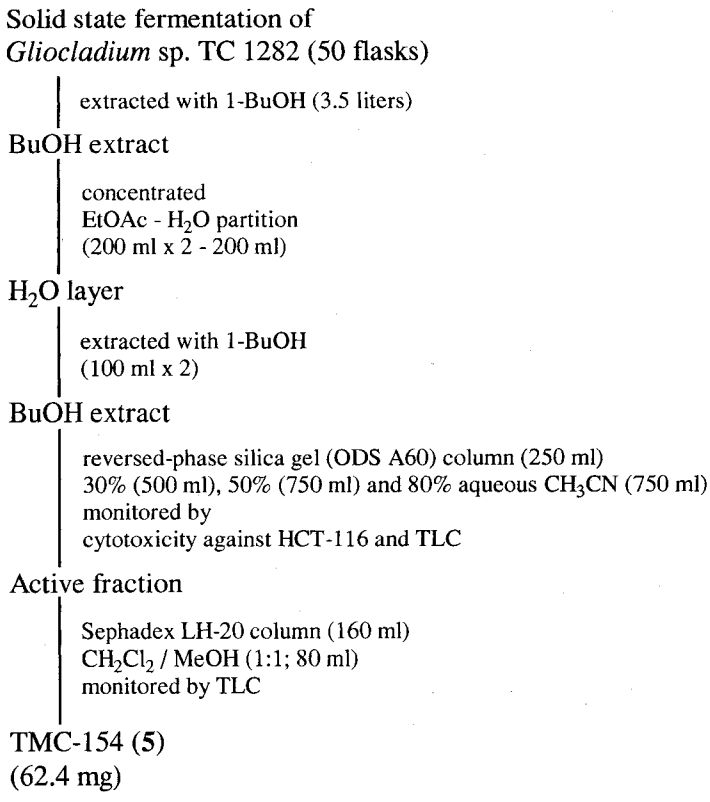
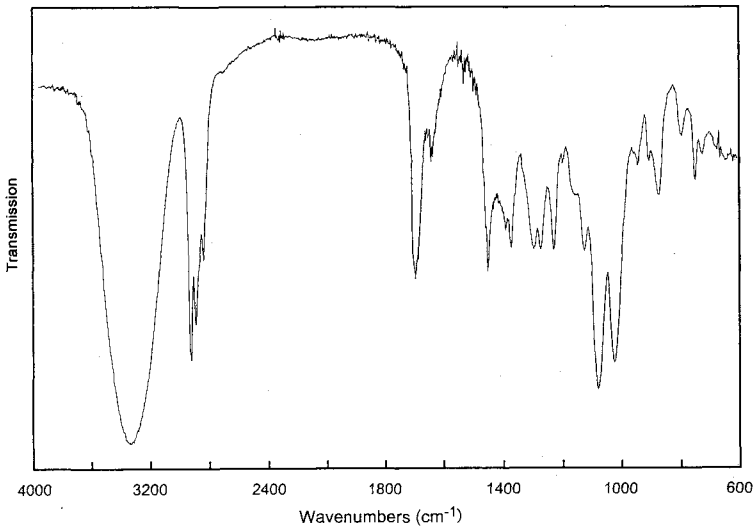


Table 1. Physico-chemical properties of TMC-171 A (**2**), B (**3**), C (**4**) and TMC-154 (**5**).

Compound	**2**	**3**	**4**	**5**
Appearance	Colorless powder	Colorless powder	Colorless powder	Colorless powder
MP (°C, dec.)	90 ~ 92	71 ~ 74	85 ~ 92	70 ~ 77
$[\alpha]_D^{24.5}$ (MeOH)	+19° (*c* 0.11)	+7° (*c* 0.03)	+24° (*c* 0.50)	+11° (*c* 0.19)
ESI-MS (*m/z*)	827 $(M+Na)^+$	827 $(M+Na)^+$	827 $(M+Na)^+$	811 $(M+Na)^+$
	803 $(M-H)^-$	803 $(M-H)^-$	803 $(M-H)^-$	787 $(M-H)^-$
HRESI-MS (*m/z*)				
Found	803.4797	803.4761	803.4802	787.4894
Calcd.	803.4793	803.4793	803.4793	787.4847
	for $C_{41}H_{71}O_{15}$	for $C_{41}H_{71}O_{15}$	for $C_{41}H_{71}O_{15}$	for $C_{41}H_{71}O_{14}$
Molecular formula	$C_{41}H_{72}O_{15}$	$C_{41}H_{72}O_{15}$	$C_{41}H_{72}O_{15}$	$C_{41}H_{72}O_{14}$
UV λ_{max} (MeOH) nm (log ε)	203 (4.42)	205 (4.40)	206 (4.36)	205 (4.75)
	222^{sh} (4.17)	222^{sh} (4.21)	220^{sh} (4.21)	220^{sh} (4.54)
IR ν_{max} (KBr) cm^{-1}	3400, 2950, 2920	3400, 2950, 2920	3400, 2950, 2920	3400, 2955, 2920
	1700, 1075, 1020	1700, 1075, 1020	1700, 1075, 1020	1700, 1075, 1020
TLC, Rf Value[a]				
EtOAc-MeOH-H_2O (15:3:1)	0.26	0.27	0.26	0.42

[a] Merck Kieselgel 60 F_{254} (Art. 5719)

Fig. 4. IR spectrum of TMC-171C (**4**) in KBr.



153.0 Hz (≪166 Hz, indicating β configuration at C-1″)[3] and ROESY correlations between H-13 and H-15, H-16 and H-27, compounds **2**~**4** were deduced to be (*E*)-14,15-dedihydro analogs of TMC-151 A~C. Their planar structures were finally confirmed by 2D-NMR experiments including DQF-COSY and HMBC as shown in Fig. 5.

TMC-154 (**5**)

The HR-ESI-MS spectral analysis of **5** gave a molecular formula of $C_{41}H_{72}O_{14}$, which differed from those of **2**~**4** by one oxygen and from that of TMC-151D ($C_{40}H_{72}O_{14}$) by one carbon. In the ^1H and ^{13}C NMR spectra for **5** (Tables 2 and 3), the spectral pattern of the alkyl chain (C-1 ~ C-20)

Table 2. ^{13}C NMR data for **2**~**5** in DMSO-d_6.

Position	2	m[a]	3	4	5
1	167.2	s	166.8	167.6	167.3
2	126.5	s	126.8	126.5	126.4
3	146.7	d	146.3	146.4	146.5
4	36.9	d	36.8	36.9	36.9
5	80.8	d	80.8	80.8	80.7
6	136.0	s	136.0	136.0	136.0
7	131.3	d	131.4	131.3	131.3
8	36.0	d	36.0	36.0	36.0
9	81.1	d	81.1	81.1	81.1
10	135.5	s	135.4	135.5	135.5
11	130.7	d	130.7	130.6	130.5
12	33.7	d	33.7	33.8	33.8
13	85.6	d	85.6	85.6	85.7
14	129.8	s	129.8	129.8	129.8
15	137.5	d	137.5	137.5	137.5
16	29.3	d	29.3	29.3	29.3
17	44.0	t	44.0	44.0	44.0
18	32.0	d	32.0	32.0	32.0
19	29.8	t	29.8	29.8	29.8
20	11.2[b]	q	11.2[c]	11.2[d]	11.1[f]
21	12.6	q	12.6	12.5	12.5
22	16.5	q	16.4	16.5	16.4
23	11.0[b]	q	11.0[c]	11.1[d]	11.2[f]
24	17.4	q	17.4	17.4	17.4
25	11.0[b]	q	11.1[c]	11.1[d]	11.2[f]
26	17.1	q	17.1	17.1	17.1
27	11.2[b]	q	11.2[c]	11.2[d]	11.2[f]
28	21.7	t	21.7	21.7	21.7
29	18.7	q	18.7	18.7	18.7
1'	63.0	t	59.8	67.2	66.0
2'	70.3	d	74.4	68.5	67.4
3'	72.5	d	67.1	69.5[e]	71.0[g]
4'	69.3	d	69.9	69.3[e]	71.2[g]
5'	70.7	d	70.9	71.1	63.5
6'	63.5	t	63.6	63.8	
1"	96.1	d	96.1	96.1	96.1
2"	70.9	d	70.9	70.9	70.3
3"	74.1	d	74.1	74.1	73.9
4"	67.3	d	67.3	67.3	77.1
5"	77.6	d	77.6	77.6	76.3
6"	61.3	t	61.3	61.3	60.8
4"-OCH_3					59.6

[a] Multiplicity.

[b-g] May be exchangeable.

closely resembled those of **2**~**4**. On the other hand, the signals corresponding to the alditol moiety (C-1'~C-5') were almost identical to that of TMC-151D. Compound **5** also showed an additional signal derived from methoxy group (δ_H 3.39, δ_C 59.6). The observation of the ^1H-^{13}C long range correlation from this methoxy group to C-4" along with the low field shift of C-4" relative to those of **2**~**4**, and $^1J_{C\text{-}1'',\ H\text{-}1''}$=153.5 Hz indicated that the sugar moiety of **5** was 4"-*O*-methyl-β-mannopyranoside. These results demonstrated the planar structure of **5** to be the 4"-*O*-methyl-(*E*)-14,15-didehydro analog of TMC-151D.

Absolute Stereochemistry

The configurations of the mannose, mannitol and arabitol moieties of **2**~**5** were all determined to be D based on the optical rotation values of the degradation products of TMC-171 complex (**1**) and TMC-154 (**5**). Methanolysis of **1** and **5** gave methyl α-D-mannopyranoside ($[\alpha]_D$ +85°, commercially available sample: $[\alpha]_D$ +80°) and methyl 4"-*O*-methyl-α-D mannopyranoside ($[\alpha]_D$ +87°), respectively. Compounds **1** and **5** were also hydrolyzed under alkaline conditions followed by acetylation (Ac_2O/pyridine) to yield hexa-*O*-acetyl-D-mannitol ($[\alpha]_D$ +24°, authentic sample[2]: $[\alpha]_D$ +26°) and penta-*O*-acetyl-D-arabitol ($[\alpha]_D$ +38°, authentic sample[2]: $[\alpha]_D$ +36°), respectively. The absolute stereochemistry at C-4, 5, 8, 9, 12, 13, 16 and 18 of **2**~**4** was determined by single crystal X-ray structure analysis of **6** obtained from the alkaline hydrolysis of **1** (Fig. 6). Since the absolute configuration of the mannoside moiety had been determined to be D, the absolute stereochemistry of **6** was established to be 4*S*, 5*S*, 8*S*, 9*S*, 12*S*, 13*S*, 16*S* and 18*S*.

Compound **7**, obtained from alkaline hydrolysis of **5**, showed almost identical ^1H and ^{13}C NMR spectra, except for the mannose moiety, with those of **6**. Moreover, the optical rotation value of **7** ($[\alpha]_D$ +15°) was similar to that of **6** ($[\alpha]_D$ +22°). TMC-154 (**5**) was thus suggested to have the same absolute stereochemistry at C-4, 5, 8, 9, 12, 13, 16 and 18 as those of **2**~**4**.

From these studies, the structures of TMC-171A (**2**), B (**3**), C (**4**) and TMC-154 (**5**) were determined as shown in Fig. 1.

Biological Activity

The cytotoxic activity of TMC-171A (**2**), B (**3**), C (**4**) and TMC-154 (**5**) against several tumor cell lines is summarized in Table 4. All compounds showed moderate cytotoxicity to various tumor cell lines.

Discussion

In this paper, we report that four new cytotoxic antibiotics, TMC-171A (**2**), B (**3**), C (**4**), and TMC-154 (**5**) were isolated from Hypocrealean fungal strains, *Gliocladium* sp. TC 1304 and TC 1282, respectively. Our structural study revealed that these antibiotics were new members of structurally unique polyketides, TMC-151s. Recently, the closely related antibiotics roselipin 1A, 1B, 2A and 2B produced by *Gliocladium roseum* KF-1040 as

Table 3. ^{1}H NMR data for **2**~**5** in DMSO-d_6.

Position	2	3	4	5
3	6.69 (1H, dd, 10, 1)[a]	6.63 (1H, dd, 10, 1)	6.67 (1H, dd, 9.8, 1.2)	6.65 (1H, dd, 9.5, 1.2)
4	2.57 (1H, m)	2.57 (1H, m)	2.57 (1H, m)	2.56 (1H, m)
5	3.68 (1H, m)	3.67 (1H, m)	3.67 (1H, m)	3.67 (1H, dd, 3.5, 8.1)
7	5.19 (1H, brd, 9)	5.19 (1H, brd, 9)	5.19 (1H, brd, 10)	5.20 (1H, d, 8.9)
8	2.44 (1H, m)	2.44 (1H, m)	2.44 (1H, m)	2.46 (1H, m)
9	3.54 (1H, m)	3.54 (1H, m)	3.54 (1H, m)	3.55 (1H, m)
11	5.25 (1H, brd, 9)	5.24 (1H, brd, 9)	5.24 (1H, brd, 10)	5.24 (1H, brd, 8.5)
12	2.56 (1H, m)	2.56 (1H, m)	2.56 (1H, m)	2.58 (1H, m)
13	3.90 (1H, d, 8.3)	3.90 (1H, d, 8.5)	3.90 (1H, d, 8.3)	3.87 (1H, d, 8.2)
15	5.12 (1H, brd, 10)	5.12 (1H, brd, 9)	5.12 (1H, brd, 10)	5.10 (1H, d, 9.1)
16	2.50 (1H, m)	2.50 (1H, m)	2.50 (1H, m)	2.50 (1H, m)
17	1.23 and 1.03 (2H, m)	1.23 and 1.03 (2H, m)	1.23 and 1.03 (2H, m)	1.22 and 1.03 (2H, m)
18	1.23 (1H, m)	1.23 (1H, m)	1.23 (1H, m)	1.23 (1H, m)
19	1.24 and 1.12 (2H, m)	1.24 and 1.12 (2H, m)	1.24 and 1.12 (2H, m)	1.24 and 1.12 (2H, m)
20	0.81 (3H, t, 7)	0.81 (3H, t, 7)	0.81 (3H, t, 7)	0.81 (3H, t, 7.1)
21	1.79 (3H, d, 1)	1.79 (3H, d, 1)	1.80 (3H, d, 1.2)	1.79 (3H, d, 1)
22	0.77 (3H, d, 6.8)	0.77 (3H, d, 6.6)	0.77 (3H, d, 6.8)	0.77 (3H, d, 6.9)
23	1.56 (3H, brs)	1.57 (3H, brs)	1.56 (3H, brs)	1.56 (3H, brs)
24	0.70 (3H, d, 6.8)	0.70 (3H, d, 6.6)	0.70 (3H, d, 6.8)	0.70 (3H, d, 6.8)
25	1.55 (3H, brs)	1.56 (3H, brs)	1.55 (3H, brs)	1.55 (3H, brs)
26	0.77 (3H, d, 6.8)	0.76 (3H, d, 6.6)	0.77 (3H, d, 6.8)	0.77 (3H, d, 6.9)
27	1.51 (3H, brs)	1.51 (3H, brs)	1.52 (3H, brs)	1.51 (3H, brs)
28	0.92 (3H, d, 6.6)	0.92 (3H, d, 6.6)	0.92 (3H, d, 6.6)	0.90 (3H, d, 6.6)
29	0.80 (3H, d, 7)	0.80 (3H, d, 7)	0.80 (3H, d, 7)	0.83 (3H, d, 7)
1'	3.39 and 3.28 (2H, m)	3.76 and 3.61 (2H, m)	4.34 and 4.00 (2H, dd)	4.06 (2H, m)
2'	3.78 (1H, m)	4.78 (1H, m)	3.72 (1H, m)	3.94 (1H, m)
3'	5.02 (1H, dd, 8, 1)	3.97 (1H, t, 8)	3.59 (1H, m)	3.27 (1H, m)
4'	3.73 (1H, m)	3.27 (1H, m)	3.58 (1H, m)	3.49 (1H, m)
5'	3.21 (1H, m)	3.48 (1H, m)	3.48 (1H, m)	3.71 and 3.48 (2H, m)
6'	3.60 and 3.39 (1H, m)	3.59 and 3.38 (2H, m)	3.62 and 3.41 (2H, m)	
1"	4.16 (1H, brs)	4.16 (1H, brs)	4.16 (1H, brs)	4.14 (1H, brs)
2"	3.54 (1H, m)	3.54 (1H, m)	3.54 (1H, m)	3.52 (1H, m)
3"	3.16 (1H, ddd, 9, 6, 3)	3.16 (1H, ddd, 9.0, 6.3, 3.4)	3.16 (1H, ddd, 9.3, 6.1, 3.2)	3.29 (1H, m)
4"	3.27 (1H, dt, 5, 9, 9)	3.27 (1H, dt, 5, 9, 9)	3.27 (1H, dt, 5.4, 9.3, 9.3)	3.11 (1H, t, 9.5)
5"	2.86 (1H, ddd, 9, 6, 2)	2.87 (1H, ddd, 9, 6, 2)	2.86 (1H, ddd, 9.3, 6.3, 2.2)	2.87 (1H, ddd, 9, 6, 3)
6"	3.67 and 3.46 (2H, m)	3.67 and 3.46 (2H, m)	3.67 and 3.45 (2H, m)	3.61 and 3.50 (2H, m)
4"-OCH_3				3.39 (3H, s)
5-OH	4.59 (1H, d, 3.9)	4.59 (1H, d, 3.9)	4.58 (1H, d, 3.7)	4.61 (1H, d, 3.9)
9-OH	4.06 (1H, d, 3.4)	4.07 (1H, d, 3.6)	4.07 (1H, d, 3.4)	4.08 (1H, d, 3.5)
1'-OH	4.42 (1H, t, 5.6)	4.53 (1H, t, 5.7)		
2'-OH	4.84 (1H, d, 5.9)		4.77 (1H, d, 6.1)	4.55 (1H, d, 6.7)
3'-OH		4.45 (1H, d, 8)	4.27 (1H, d, 7.6)	4.43 (1H, d, 7.5)
4'-OH	4.63 (1H, d, 6.3)	4.25 (1H, d, 7.6)	4.13 (1H, d, 7.6)	4.48 (1H, d, 5.5)
5'-OH	4.27 (1H, d, 5.4)	4.44 (1H, d, 5)	4.41 (1H, d, 5.4)	4.32 (1H, m)
6'-OH	4.27 (1H, t, 5.7)	4.30 (1H, t, 5.5)	4.31 (1H, t, 5.6)	
2"-OH	3.87 (1H, d, 4.4)	3.88 (1H, d, 4.6)	3.84 (1H, d, 4.4)	4.00 (1H, d, 4.7)
3"-OH	4.49 (1H, d, 6.3)	4.50 (1H, d, 6.3)	4.49 (1H, d, 6.1)	4.67 (1H, d, 7.1)
4"-OH	4.65 (1H, d, 5.6)	4.66 (1H, d, 5.4)	4.65 (1H, d, 5.4)	
6"-OH	4.34 (1H, t, 5.9)	4.35 (1H, t, 6.3)	4.33 (1H, t, 6)	4.47 (1H, m)

[a] Proton number, multiplicity and coupling constants in Hz.

Fig. 5. 2D NMR experiments of TMC-171C (**4**).

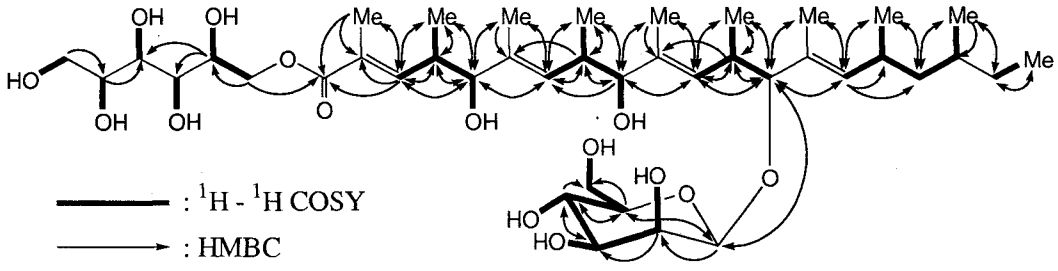


Fig. 6. *ORTEP* II diagram of **6**.

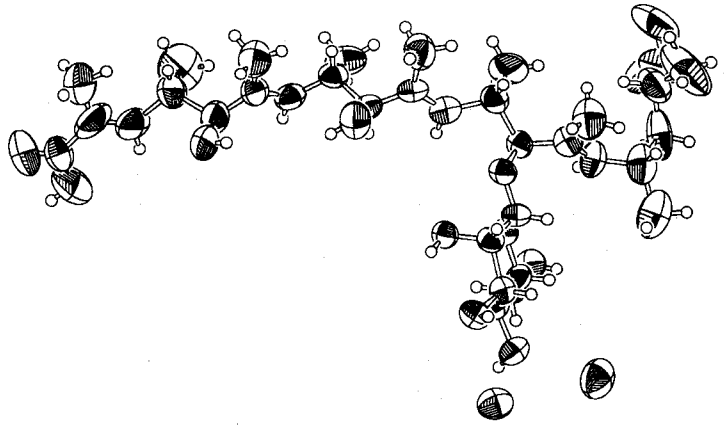


Table 4. Cytotoxicity of **2**~**5** and TMC-151C against several tumor cell lines *in vitro*.

Cell line	IC_{50} (μM)				
	2	**3**	**4**	**5**	TMC-151C
HCT-116 human colon carcinoma	65	65	60	60	4
B16 mouse melanoma	51	33	27	31	7
HeLa human cervix adenocarcinoma	62	62	62	63	9
SK-Br-3 mammary gland adenocarcinoma	70	61	59	54	17
WiDr human colon adenocarcinoma	61	62	64	63	13
HL-60 human promyelocytic leukemia	64	63	64	34	11
Jurkat human lymphoma	62	62	64	6	5
P388D1 lukemia	16	17	16	8	4
U937 human histiocytic lymphoma	11	8	15	7	7

acyl-CoA : 1,2-diacyl-*sn*-glycerol *O*-acyltransferase inhibitors, have been reported by ŌMURA *et al.*[4]

TMC-151s, TMC-154, and TMC-171s as well as roselipins are all derived from *Gliocladium* sp. It is thus an attractive issue to find out the relationship between taxonomic characteristics and production of these compounds. A number of *Gliocladium* strains and related genera were therefore collected and examined. The details

will be reported in a separate paper.[5]

Experimental

General

Rf values were determined with Kieselgel 60 F_{254} TLC glass plates (E. Merck, Darmstadt, Germany) in EtOAc/MeOH/H_2O (15 : 3 : 1). Melting points were obtained using a Yanaco MP-500D micro melting apparatus and are uncorrected. Optical rotations were determined using the sodium D line on a Horiba model SEPA-200 high sensitive polarimeter. UV spectra were measured on a Shimadzu model UV-2200A spectrophotometer. IR spectra were recorded on a JASCO model 100 infrared spectrophotometer. All mass spectra were obtained using MStation 700 tandem type mass spectrometer (JEOL, Japan) equipped with an electrospray ionization source. Analytical HPLC were obtained using a HP1100 system (Hewlett Packard, USA). The ^1H and ^{13}C NMR spectra were recorded on a JEOL GSX-400 NMR spectrometer at 30°C. The chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as an internal standard.

Fermentation

Gliocladium sp. TC 1282 and TC 1304 were inoculated into 500-ml Erlenmeyer flasks containing pressed barley 10 g, yeast extract 0.02 g, Na tartrate 0.01 g, KH_2PO_4 0.01 g, and deionized water 20 ml. The fermentation was conducted under the static condition at 25°C for 12 days.

Methanolysis of TMC-171 Complex (1)

A solution of 200 mg of **1** in 0.83 N methanolic hydrogen chloride (20 ml) was refluxed for 1.5 hours. After evaporation of the solvent *in vacuo*, the residue was dissolved in H_2O (20 ml), washed with diethyl ether (20 ml, twice), neutralized with 1 N NaOH and then the solution was concentrated to dryness to give a crude solid. This solid was subjected to a silica gel column (2.2×69 cm) and developed with EtOAc - MeOH - H_2O (20 : 3 : 1). The eluate was concentrated and chromatographed on a Sephadex LH-20 column (2.2×42 cm) with 50% aqueous MeOH as an eluent to yield amorphous powder of methyl α-D-mannopyranoside (27.0 mg): $[\alpha]_D^{27}$ +85° (*c* 0.22, MeOH); ESI-MS *m/z* 217 $(M+Na)^+$, 193 $(M-H)^-$; ^{13}C NMR data was identical with that of the authentic sample.[2]

Methanolysis of TMC-154 (5)

TMC-154 (**5**) (140 mg) was treated with methanolic hydrogen chloride as described above to yield methyl 4-*O*-methyl-α-D-mannopyranoside (17.7 mg): $[\alpha]_D^{27}$ +87° (*c* 0.06, MeOH); ESI-MS *m/z* 231 $(M+Na)^+$, 207 $(M-H)^-$; ^{13}C NMR (DMSO-d_6) δ 100.8 (C-1), 76.8 (C-4), 72.4 (C-5), 70.8 (C-2), 70.5 (C-3), 60.7 (C-6), 60.0 (CH_3O-4), 53.9 (CH_3O-1).

Alkaline Hydrolysis of TMC-171 Complex (1)

Fifteen milliliters of 0.4 N aqueous KOH was added to **1** (411 mg), and stand at room temperature for 2 hours. The reaction mixture was then neutralized with 1 N HCl. The resulting suspension was filtered and the residual solid was dried under reduced pressure to give 407 mg of the crude product. This product was purified by column chromatography on a silica gel (EtOAc - MeOH - H_2O= 30 : 3 : 1 as an eluent) to give **6** (265 mg). Compound **6** was recrystallized from MeOH to afford colorless needle.

6: $[\alpha]_D^{27}$ +22° (*c* 0.42, MeOH); Rf value 0.48; ESI-MS *m/z* 663 $(M+Na)^+$, 679 $(M+K)^+$, 639 $(M-H)^-$; HRESI-MS *m/z* 639.4089 $(M-H)^-$, (calcd for $C_{35}H_{59}O_{10}$ 639.4108); ^1H NMR (DMSO-d_6) δ 6.60 (H-3, dd, 9.8, 1.2 Hz), 5.24 (H-11, d, 8.3 Hz), 5.19 (H-7, d, 9.3 Hz), 5.12 (H-15, d, 9.8), 4.60 (5-OH, d, 3.7 Hz), 4.16 (H-1″, s), 4.01 (9-OH, br s), 3.90 (H-13, d, 8.3), 3.87 (2″-OH, br s), 3.67 (H-6″a, m), 3.67 (H-5, m), 3.54 (H-9, m), 3.54 (H-2″, m), 3.46 (H-6″b, dd, 11.6, 6.5 Hz), 3.27 (H-4″, t, 9.3 Hz), 3.16 (H-3″, dd, 9.3, 3.2 Hz), 2.87 (H-5″, ddd, 9.3, 6.5, 2.0 Hz), 2.58 (H-12, m), 2.56 (H-4, m), 2.51 (H-16, m), 2.44 (H-8, m), 1.74 (H-21, d, 1.0 Hz), 1.55 (H-23, br s), 1.55 (H-25, br s), 1.51 (H-27, br s), 1.24 (H-19a, m), 1.2 (H-18, m), 1.23 (H-17a, m), 1.12 (H-19b, m), 1.03 (H-17b, m), 0.92 (H-28, d, 6.6 Hz), 0.81 (H-20, t, 7 Hz), 0.80 (H-29, d, 7 Hz), 0.78 (H-22, d, 6.8 Hz), 0.77 (H-26, d, 6.8 Hz), 0.70 (H-24, d, 6.8 Hz); ^{13}C NMR (DMSO-d_6) δ 169.0 (C-1), 145.7 (C-3), 137.6 (C-15), 136.2 (C-6), 135.5 (C-10), 130.9 (C-7), 130.6 (C-11), 129.9 (C-14), 126.8 (C-2), 96.1 (C-1″), 85.6 (C-13), 81.1 (C-9), 80.5 (C-5), 77.7 (C-5″), 74.1 (C-3″), 70.9 (C-2″), 67.3 (C-4″), 61.3 (C-6″), 44.0 (C-17), 36.9 (C-4), 36.1 (C-8), 33.8 (C-12), 32.0 (C-18), 29.8 (C-19), 29.3 (C-16), 21.7 (C-28), 18.7 (C-29), 17.4 (C-24), 17.1 (C-26), 16.5 (C-22), 12.4 (C-21), 11.3, 11.2, 11.2 and 11.1 (C-20, 23, 25 and 27).

The filtrate of the reaction mixture described above was concentrated and the product was acetylated in the following manner.

Acetylation of the Alkaline Hydrolysis Product of 1

To a solution of the product in dry pyridine (8.0 ml) was added acetic anhydride (2.0 ml), and the mixture was stand at room temperature for 2 hours. After completion of the reaction, EtOAc (100 ml) was added, and the mixture was

washed with H_2O (50 ml, three times). The organic phase was evaporated under reduced pressure, and was chromatographed over a Sephadex LH-20 (2.2×42 cm) column in CH_2Cl_2-MeOH (1 : 1). Elution with the same solvent system afforded 140 mg of colorless powder. This powder was recrystallized from hot MeOH to afford 48 mg of hexa-*O*-acetyl-D-mannitol: Colorless plates; $[\alpha]_D^{25}$ +24° (*c* 0.50, $CHCl_3$); ESI-MS *m/z* 457 $(M+Na)^+$, 473 $(M+K)^+$; ^1H and ^{13}C NMR data were identical with those of the authentic sample.[2)]

Alkaline Hydrolysis of TMC-154 (**5**)

TMC-154 (**5**) (127 mg) was hydrolyzed with aqueous KOH as described above to give **7** (63 mg) as colorless powder.

7: $[\alpha]_D^{27}$ +15° (*c* 0.14, MeOH); ESI-MS *m/z* 677 $(M+Na)^+$, 653 $(M-H)^-$; HRESI-MS *m/z* 653.4307 $(M-H)^-$, (calcd for $C_{36}H_{61}O_{10}$ 653.4264); ^1H NMR (DMSO-d_6) δ 6.60 (H-3, dd, 9.8, 1.2 Hz), 5.24 (H-11, d, 8.0 Hz), 5.19 (H-7, d, 9.3 Hz), 5.10 (H-15, d, 9.3 Hz), 4.60 (5-OH, d, 2.9 Hz), 4.14 (H-1″, s), 4.01 (9-OH, br s), 3.87 (H-13, d, 8.3 Hz), 3.67 (H-5, br d), 3.62 (H-6″a, br d), 3.55 (H-9, m), 3.52 (H-2″, m), 3.49 (H-6″b, dd, 11.6, 5.7 Hz), 3.39 (4″-OCH_3, s), 3.30 (H-3″, dd, 9.0, 3.2 Hz), 3.11 (H-4″, t, 9.3 Hz), 2.87 (H-5″, m), 2.58 (H-12, m), 2.56 (H-4, m), 2.53 (H-16, m), 2.44 (H-8, m), 1.74 (H-21, d, 1.3 Hz), 1.56 (H-23, s), 1.55 (H-25, s), 1.51 (H-27, s), 1.22 (H-17a, m), 1.22 (H-19a, m), 1.12 (H-19b, m), 1.03 (H-18, m), 0.90 (H-28, d, 6.6 Hz), 0.82 (H-17b, m), 0.83 (H-29, d, ~7 Hz), 0.80 (H-20, d, 7.1 Hz), 0.78 (H-22, d, 6.9 Hz), 0.77 (H-26, d, 6.9 Hz), 0.70 (H-24, d, 6.8 Hz); ^{13}C NMR (DMSO-d_6) δ 169.0 (C-1), 145.7 (C-3), 137.6 (C-15), 136.2 (C-6), 135.5 (C-10), 130.9 (C-7), 130.6 (C-11), 129.9 (C-14), 126.8 (C-2), 96.1 (C-1″), 85.8 (C-13), 81.0 (C-9), 80.5 (C-5), 77.1 (C-5″), 76.3 (C-4″), 73.9 (C-3″), 71.2 (C-2″), 60.8 (C-6″), 59.6 (OCH_3), 44.0 (C-17), 36.9 (C-4), 36.0 (C-8), 33.8 (C-12), 32.0 (C-16), 29.8 (C-19), 29.3 (C-18), 21.7 (C-28), 18.7 (C-29), 17.4 (C-24), 17.1 (C-26), 16.5 (C-22), 12.4 (C-21), 11.3, 11.2, 11.2 and 11.1 (C-20, 23, 25 and 27).

Acetylation of the Alkaline Hydrolysis Product of **5**

The filtrate of the reaction mixture described above was concentrated and the product was acetylated in the similar manner described above to afford penta-*O*-acetyl-D-arabitol (20 mg): $[\alpha]_D^{25}$ +38° (*c* 0.17, $CHCl_3$); ESI-MS *m/z* 485 $(M+Na)^+$, 380 $(M+NH_4)^+$; ^1H and ^{13}C NMR data were identical with those of the authentic sample.[2)]

X-ray Crystallography of **6**

A colorless needle of **6** with dimensions 0.40×0.20×0.05 mm was used for X-ray analysis. The intensity data were collected on a Rigaku AFC5R difractometer by using graphite-monochromated Cu-Kα (λ=1.5418 Å) radiation by $2\theta/\omega$ scan technique. Unit cell dimensions were determined by a least squares refinement by using the setting of 25 reflections in the range of $70°<2\theta<90°$. The crystallographic data are summarized as follows: $C_{35}H_{60}O_{10}\cdot 2H_2O$, Mr=676.86, plate, $P2_1$, a=16.539(3) Å, b=7.653(3) Å, c=16.687(3) Å, V=2076.5(9) Å^3, Z=2, D_{calc}=1.08 g/cm^3, Mu=0.658 mm^{-1}. The intensities of 6326 reflections with $2.7°<\theta<65.1°$, $-10<h<19$, $-4<k<9$, $-19<l<19$ were measured. The data were corrected for Lorentz and polarization effects, but not for absorption.

The structure was solved by a direct method by using SHELXS-97[6)] and the subsequent difference Fourier method. The structure refinement on F^2 was carried out by a SHELXL-97[7)] with anisotropic thermal parameters for all of non-hydrogen atoms. The molecule have disordered part at the end of alkyl chain with occupancy factor of 0.46 and 0.54. The hydrogen atoms were refined by riding with the atoms to which they were bonded. The full matrix least squares refinement varied 434 parameters and used all 4324 independent reflections weighted by $\omega=1/[\sigma^2(Fo^2)+(0.1000P)^2+0.0000P]$ where $P=(Fo^2+2Fc^2)/3$. Final $R1$=0.072 for 2074 reflections with $I>2(I)\sigma$ and *Goodness of Fit*(S)=1.21; $R1$=0.157, $wR2$=0.230 for all data;. The final difference Fourier map showed maximum and minimum values of 0.55 and $-0.23e$/Å^3, respectively.

In Vitro Cytotoxic Activity

The cells used for assay were cultured in the following medium; HCT-116 and SK-Br-3: complete McCoy's 5A supplemented with 10% fetal bovine serum, B16 and HeLa: complete D-MEM supplemented with 10% fetal bovine serum, HL-60: complete RPMI-1640 supplemented with 20% fetal bovine serum, WiDr: complete RPMI-1640 supplemented with non essential amino acid solution and 10% fetal bovine serum, Jurkat and U937: complete RPMI-1640 supplemented with 1% fetal bovine serum, P388D_1: complete RPMI-1640 supplemented with 5% fetal bovine serum.

In vitro cytotoxic activity was tested in 96-well microtiter plates of which well containing 1×10^4 each cell lines in 135 μl medium. The test samples were dissolved in 10% DMSO. The serially diluted DMSO solution (15 μl) was added to each well of plates. After addition, the cells were incubated at 37°C for 72 hours in a humidified 5% CO_2 atmosphere. In vitro cytotoxic activity was evaluated by the microculture tetrazolium assay (MTT assay) method for

each cell and by the colorimetrical determination method at 540 nm.

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