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TMC-169, a New Antibiotic of the Aspochalasin Group Produced by *Aspergillus flavipes*

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In the course of screening for new bioactive compounds, a new antibiotic has been isolated from a fungal strain *Aspergillus flavipes* TC 1446 and was designated TMC-169. Spectroscopic analyses revealed that TMC-169 was a new analog of the aspochalasin group, represented by aspochalasins A~G[1~3] and phomacin C[4]. This paper briefly describes the taxonomy, isolation, physico-chemical properties, structure elucidation and biological activity of TMC-169.

The producing strain TC1446, a soil isolate, was identified as *Aspergillus flavipes* (Bain. & Sart.) Thom & Church on the basis of the following distinct characteristics: grayish orange to brownish yellow conidial area, a number of pseudo-sclerotia embedded in hülle cells, loosely columnar biseriate aspergilla on long brownish stipes, and smooth-walled subglobose to ellipsoidal conidia of average diameter 2.5 μm (2.0~2.4 × 1.5~3.0 μm). *Aspergillus flavipes* is anamorph of *Fennelia flavipes*, although the sclerotia of our strain did not mature within two months.

TMC-169 was produced by solid state fermentation in a 500-ml Erlenmeyer flask 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 static conditions at 25°C for 12 days. The resultant culture of three flasks was extracted with 1-butanol (210 ml) by shaking for 30 minutes. The extract was concentrated and then dissolved in water (60 ml) and extracted twice by ethyl acetate (60 ml). The ethyl acetate layer was concentrated, dissolved in 90% aqueous methanol (60 ml), and partitioned against *n*-hexane (60 ml). The aqueous methanol layer was concentrated to dryness under a reduced pressure, giving a crude solid (840 mg). This solid was purified by gel filtration on a Sephadex LH-20 column developed with CH_2Cl_2-CH_3OH (1:1). The eluate was monitored by bioassay for cytotoxic activity against HCT-116[5] and silica gel TLC (CH_2Cl_2-CH_3OH (9:1), Rf=0.60). The active fractions were concentrated and subjected to reverse phase silica gel (YMC ODS A60) column chromatography, followed by stepwise elution with 45 and 60% aqueous acetonitrile. The active fractions eluted with 60% aqueous acetonitrile were concentrated to give a semi-pure solid (78 mg). Final purification of TMC-169 was achieved by preparative HPLC (column, YMC-D-ODS-5 30 × 250 mm; mobile phase, 50% aqueous acetonitrile; flow rate, 25 ml/minute; detection, UV 243 nm; Rt, 42 minutes) followed by Sephadex LH-20 column chromatography. Pure TMC-169 (27 mg) was obtained as a colorless powder, and the physico-chemical properties are listed in Table 1.

The molecular formula of TMC-169 was established as $C_{24}H_{35}NO_3$ on the basis of high-resolution ESI-MS, and ^1H and ^{13}C NMR spectral data. The IR absorption at 1680 cm^{-1} together with the UV absorption at 245 nm indicated the presence of an α,β-unsaturated ketone. The presence of the amide group was also suggested by the IR absorption at 3400 and 1680 cm^{-1}.

The ^{13}C and ^1H NMR data of TMC-169 summarized in Table 2 were obtained from ^1H, ^{13}C, DEPT, and pulsed field gradient (PFG)-HMQC spectra.

The ^{13}C and ^1H NMR spectra displayed 24 carbon and 34 proton signals, respectively, and suggested the presence of one amide carbonyl (δ_C 174.6), one α,β-unsaturated carbonyl (δ_C 197.8), six olefinic sp^2 carbons (δ_C 124.8~145.2), and two high deshielded methyl protons (δ_H 1.77 and 1.39). The DQF-COSY spectrum showed the four partial structures graphically

Fig. 1. Structure of TMC-169.

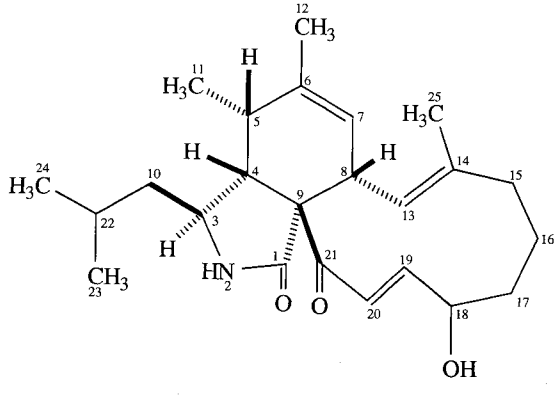


Table 1. Physico-chemical properties of TMC-169.

Appearance	Colorless powder
MP	103 ~ 105°C
$[\alpha]_D^{23}$	-50°(*c* 0.31, MeOH)
ESI-MS (*m/z*)	386 $(M+H)^+$, 384 $(M-H)^-$
HRESI-MS (*m/z*)	
Found	384.2507 $(M-H)^-$
Calcd.	384.2539 for $C_{24}H_{34}NO_3$
Molecular formula	$C_{24}H_{35}NO_3$
UV λmax (MeOH) nm	245 sh
IR ν_{max} (KBr) cm^{-1}	3400, 2950, 2925, 1680, 1620
	1450, 1380, 1240, 1220

Table 2. ^{13}C and ^1H NMR data for TMC-169[a].

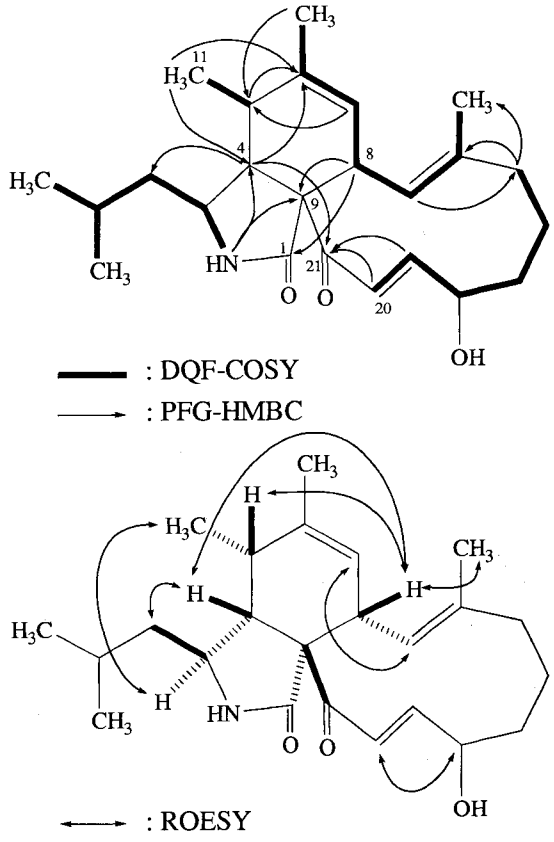
No.	δ_C	δ_H
1	174.6 s[b]	
2		6.11 (1H, brs)[c]
3	51.0 d	3.12 (1H, m)
4	49.4 d	3.06 (1H, m)
5	35.0 d	2.48 (1H, m)
6	140.4 s	
7	125.9 d	5.43 (1H, m)
8	43.6 d	2.86 (1H, m)
9	67.8 s	
10	48.4 t	1.24 (2H, m)
11	13.6 q	1.24 (3H, d, 7.1)
12	20.0 q	1.77 (3H, d, 1.2)
13	124.8 d	6.00 (1H, brd, 11)
14	138.1 s	
15	40.6 t	2.10 (1H, m)
		1.99 (1H, m)
16	20.9 t	1.82 (1H, m)
		1.60 (1H, m)
17	37.9 t	2.02 (1H, m)
		1.83 (1H, m)
18	72.1 d	4.43 (1H, m)
19	145.2 d	6.55 (1H, dd, 16.2, 5.9)
20	128.7 d	7.22 (1H, dd, 16.2, 1.2)
21	197.8 s	
22	25.1 d	1.56 (1H, m)
23	23.7 q	0.90 (3H, d, 6.6)
24	21.4 q	0.89 (3H, d, 6.6)
25	17.5 q	1.39 (3H, d, 1.2)

[a] Recorded in $CDCl_3$ at 100 and 400 MHz, respectively

[b] Multiplicity.

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

Fig. 2. 2D NMR experiments of TMC-169.



illustrated as bold lines (Fig. 2). The connectivity of these units was established by PFG-HMBC experiments. The key long range couplings from NH-1 (δ 6.11) and H-8 (δ 2.86) to quaternary carbon C-9 (δ 67.8), from NH-1 and H-11 (δ 1.24) to methine carbon C-4 (δ 49.4), and from H-8 to amide carbon C-1 (δ 174.6) constructed the isoindole structure. Both of double bonds at C-13 and C-19 were assigned as *E* configuration based on the observation of ROE between H-8 and H-25, and the large vicinal coupling constant ($^3J_{19,20}$ = 16.2 Hz). In addition, the relative stereochemical assignment of the isoindole moiety was made by a ROESY experiment. The cross peaks from H-8 to H-4 (δ 3.06) and H-5 (δ 2.48), and from H-4 to H-10 indicated that H-4, H-5, H-8 and isobutyl unit (C-10, 22, 23 and 24) were on the same side of the isoindole ring. On the other hand, the signal of H-3 showed a cross peak with that of the methyl proton at C-5, indicating that H-3 and the methyl group at C-5 are both on the opposite side of the ring. These results lead the structure of TMC-169 as illustrated in Fig. 1.

Table 3. Cytotoxicities of TMC-169 against tumor cells *in vitro*.

Cell lines	IC_{50} (μM)
U937 human histiocytic lymphoma	0.81
Jurkat human lymphoma	0.21
HL-60 human promyelocytic leukemia	0.68
WiDr human colon adenocarcinoma	0.83
HCT-116 human colon carcinoma	0.78

The determined structure closely resembles those of aspochalasin group antibiotics. The relative stereochemistry of the isoindole moiety of TMC-169 is the same as those of aspochalasins. The assigned ^{1}H and ^{13}C NMR data of TMC-169 well corresponded to those of phomacin C[4] except for the signals at C-16. TMC-169 is thus considered to be a 17-deoxy analog of aspochalasin C or D, and a 16-dehydroxymethyl analog of phomacin C, although the stereochemistry of TMC-169 at C-18 has not yet been clarified.

The cytotoxic activity of TMC-169 against several tumor cell lines, determined according to the methods previously descrived[5], is summarized in Table 3. The compound showed moderate cytotoxicities to various tumor cell lines.

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