TMC-34, a New Macrolide Antifungal Antibiotic

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In the course of screening for antifungal agents active against *Candida albicans*, a new antifungal antibiotic has been isolated from an actinomycete strain A-3030 and was designated TMC-34. Spectroscopic analysis revealed that TMC-34 belonged to the class of 32-membered macrolide antibiotics represented by copiamycin[1,2] and neocopiamycin[3]. This paper describes the production, isolation, physico-chemical properties, structure elucidation and biological activity of TMC-34.

The producing strain A-3030 was isolated from a soil sample collected in Fukuchiyama, Kyoto Prefecture, Japan, and taxonomic studies indicated that the strain belonged to the genus *Streptomyces*. This strain was cultured in 500-ml Erlenmeyer flasks containing 70 ml of a production medium containing glucose 0.5%, soluble starch 2.0%, glycerol 2.0%, soybean meal 2.0%, yeast

Fig. 1. Isolation and purification of TMC-34.

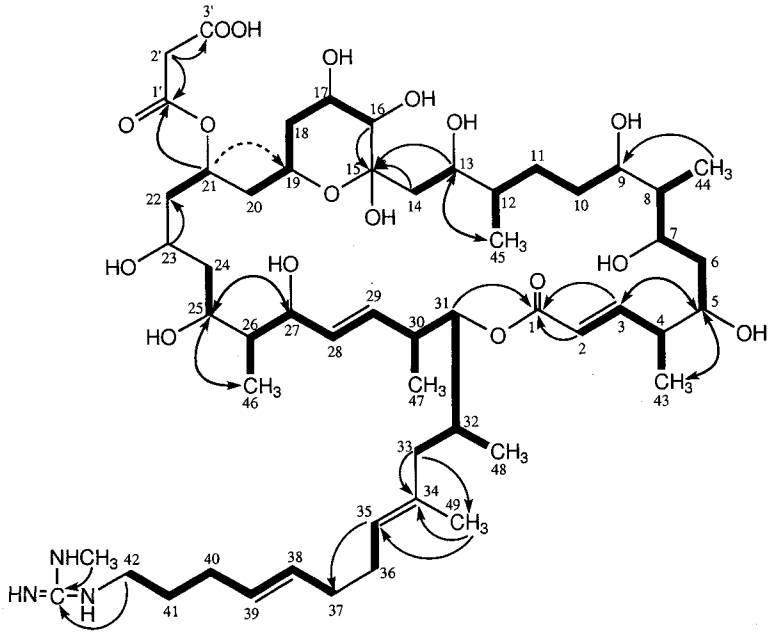
Whole broth (3.5 liters)
↓ extracted with 1-BuOH (1.5 liters)
BuOH extract (800 ml)
↓ concentrated
EtOAc - H_2O partition
(250 ml x 2 -250 ml)
H_2O layer
↓ extracted with 1-BuOH (250 ml x 2)
BuOH layer
↓ concentrated
washed with acetone (100 ml x 2)
Precipitation
↓ extracted with MeOH (100 ml x 2)
concentrated
Crude solid (892 mg)
↓ reversed phase silica gel (ODS A60) column chromatography
eluted with 45% CH_3CN - H_2O
Semi-pure solid (327 mg)
↓ 1) preparative HPLC on YMC D-ODS-5 column
eluted with 40% CH_3CN - 10 mM phosphate buffer (pH 3.5)
2) Sephadex LH-20 column chromatography
eluted with MeOH
3) crystallized from hot aqueous acetone
TMC-34 (64.7 mg)

Table 1. ^{13}C and ^{1}H NMR data of TMC-34[a].

No.	^{13}C (δ)	^{1}H (δ)	No.	^{13}C (δ)	^{1}H (δ)
1	168.2 (s)		25	68.9 (d)	4.14 (1H, m)
2	123.3 (d)	5.89 (1H, d, 15.6)[b]	26	45.5 (t)	1.45 (1H, m)
3	152.5 (d)	6.92 (1H, dd, 15.6, 9.0)	27	75.8 (d)	3.88 (1H, m)
4	43.4 (d)	2.47 (1H, m)	28	134.7 (d)	5.47 (1H, m)
5	75.3 (d)	3.75 (1H, m)	29	134.9 (d)	5.54 (1H, m)
6	39.4 (t)	1.46 (1H, m)	30	40.1 (d)	2.52 (1H, m)
		1.51 (1H, m)	31	80.3 (d)	4.75 (1H, dd, 7.0, 4.3)
7	74.8 (d)	3.75 (1H, m)	32	33.5 (d)	2.00 (1H, m)
8	44.6 (d)	1.52 (1H, m)	33	45.2 (t)	1.77 (2H, m)
9	72.1 (d)	3.91 (1H, m)	34	134.1 (s)	
10	33.4 (t)	1.56 (1H, m)	35	127.7 (d)	5.13 (1H, m)
		1.67 (1H, m)	36	29.0 (t)	2.09 (2H, m)
11	30.6 (t)[c]	1.38 (1H, m)	37	33.7 (t)	2.08 (2H, m)
		1.45 (1H, m)	38	132.5 (d)	5.54 (1H, m)
12	40.7 (d)	1.61 (1H, m)	39	130.1 (d)	5.45 (1H, m)
13	72.6 (d)	3.84 (1H, m)	40	30.7 (t)[c]	2.09 (2H, m)
14	41.3 (t)	1.83 (2H, m)	41	29.8 (t)	1.65 (2H, m)
15	99.7 (s)		42	41.9 (t)	3.16 (2H, t, 7.1)
16	77.1 (d)	3.36 (1H, d, 9.1)	43	16.5 (q)	1.13 (3H, d, 6.9)
17	69.7 (d)	3.89 (1H, m)	44	10.5 (q)	0.88 (3H, d, 7.0)
18	41.6 (t)	1.30 (1H, m)	45	14.5 (q)	0.91 (3H, d, 6.9)
		1.90 (1H, ddd)	46	11.2 (q)	0.79 (3H, d, 7.0)
19	65.6 (d)[d]	4.11 (1H, m)	47	17.8 (q)	0.98 (3H, d, 6.9)
20	42.0 (t)	1.67 (1H, m)	48	14.5 (q)	0.86 (3H, d, 6.6)
		1.72 (1H, m)	49	16.3 (q)	1.58 (3H, brs)
21	70.9 (d)	5.22 (1H, m)	guanido	158.3 (s)	
22	44.7 (t)	1.73 (1H, m)	NCH_3	28.3 (q)	2.82 (3H, s)
		1.80 (1H, m)	1'	171.6 (s)	
23	65.7 (d)[d]	3.88 (1H, m)	2'	46.9 (t)	3.23 (2H, s)
24	43.2 (t)	1.55 (1H, m)	3'	174.1 (s)	
		1.62 (1H, m)			

[a] ^{13}C NMR (100 MHz) and ^{1}H NMR (400 MHz) were measured in CD_3OD at 30 ℃.
[b] Proton number, multiplicity and coupling constants in Hz are indicated in parenthese.
[c,d] May be exchangeable.

Fig. 2. Structure of TMC-34.



: ^{1}H-^{1}H couplings obtained from ^{1}H-^{1}H COSY,
: long-range couplings obtained from HMBC and selective INEPT,
: ^{1}H-^{1}H coupling obtained from HOHAHA.

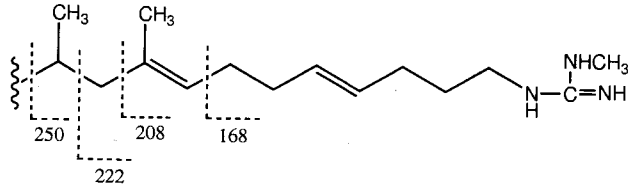
extract 0.2% and $CaCO_3$ 0.3% (pH 7.0 before autoclaving). The flasks were incubated at 27°C for 5 days on a rotary shaker at 220 rpm. The production of the antibiotic was monitored by antifungal activity against *C. albicans* ATCC 48130.

The isolation procedure for TMC-34 is summarized in Fig. 1. TMC-34 was extracted from the fermentation broth with 1-butanol and was purified by solvent partition, precipitation and repeated column chromatography followed by crystallization from hot aqueous acetone.

TMC-34 was obtained as white fine needles and was soluble in dimethyl sulfoxide, pyridine and lower alcohols but hardly soluble in other organic solvents and water. TMC-34: MP 146~148°C (dec); $[\alpha]_D^{20}$ +18° (*c* 0.29, MeOH); UV λ_{max}^{MeOH} nm (ε) 205 (17,600); IR ν_{max}^{KBr} cm^{-1} 3380, 2970, 2940, 1710, 1650, 1600, 1380, 1290, 1245, 1145, 1070, 980; FAB-MS *m/z* 1,056 $(M+H)^+$; Elemental analysis, Calcd for $C_{54}H_{93}N_3O_{17}\cdot 2H_2O$: C 59.37, H 8.95, N 3.85, Found: C 59.03, H 8.92, N 3.70; Positive color reaction: iodine vapor and ammonium molybdate-sulfic acid reagents; Negative color reaction: ninhydrin reagent; Rf 0.09 (silica gel, Merck Art. No. 5715, l-butanol - acetic acid - water (3 : 1 : 1)).

TMC-34 showed an absorption maximum at 205 nm in the UV spectrum, suggesting the presence of α,β-unsaturated ester group in this structure. The IR spectrum suggested the presence of a guanidine group (1650 and 1600 cm^{-1}). The molecular formula of TMC-34 was determined as $C_{54}H_{93}N_3O_{17}$ based on FAB-MS, ^{1}H and ^{13}C NMR and elemental analysis data.

Fig. 3. FAB-MS fragmentations of TMC-34.



The ^{1}H and ^{13}C NMR data of TMC-34 are shown in Table 1. The ^{13}C NMR spectrum displayed 54 signals composed of CH_3–C×7, CH_3–N×1, CH_2–×16, >CH–×17, >C<×1, CH=×7, >C=×2 and carbonyl C×3. The structure of TMC-34 was determined based on the C-H connectivities which was elucidated by ^{1}H-^{1}H COSY, HMQC, HMBC, selective INEPT and HOHAHA spectra (Fig. 2). The position of C-34 double bond was confirmed by the fragment ion *m/z* 168 and 208 of the FAB-MS as shown in Fig. 3. The configuration of the double bond was revealed to be *E* by the observation of NOE between 36-H and the protons of 49-CH_3. The position of the hemiester (C-1′) was assigned to C-21 based on the observation of long range couplings between H-21-C-1′ and H-21-H-19.

The determined structure of TMC-34 closely resembles those of copiamycin[1,2] and neocopiamycin[3]. TMC-34 is considered to be 34,35-dedihydro analog of copiamycin, although the position of malonic hemiester has not been decided in copiamycin.

Table 2. Antimicrobial spectra of TMC-34.

Test organisms	MIC (μg/ml)
Candida albicans ATCC 48130	3.1
Cryptococcus neoformans 145A	1.6
Aspergillus fumigatus TUKUBA	3.1
Trichophyton mentagrophytes	3.1
Trichophyton rubrum	1.6
Staphylococcus aureus 209P JC-1	>100
Staphylococcus epidermidis Kawamura	>100
Enterococcus faecalis ATCC 29212	>100
Bacillus subtilis ATCC 6633	>100
Esherichia coli NIHJ JC-2	>100
Klebsiella pneumoniae PCI-602	>100
Proteus vulgaris IID 874	>100
Morganella morganii Kono	>100
Pseudomonas aeruginosa 35R	>100

MIC values were determined by agar dilution method.
For yeasts and fungi: 10^6 cells or spores/ml, 27°C.
For bacteria: 10^6 cfu/ml, 37°C.

The antimicrobial spectrum of TMC-34 is shown in Table 2. TMC-34 was active against yeasts and filamentous fungi but not active against bacteria. TMC-34 showed no cytotoxicity against human colon carcinoma HCT-116 and human leukemia HL-60 at 5.0 μg/ml.

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