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TMC-264, a Novel Antiallergic Heptaketide Produced by the Fungus *Phoma* sp. TC 1674

Masaaki Sakurai, Maki Nishio,* Kouzou Yamamoto, Toru Okuda,[†] Kimio Kawano, and Tetsuo Ohnuki

Discovery Research Laboratory, Tanabe Seiyaku Co., Ltd., 2-50 Kawagishi-2-chome, Toda-shi, Saitama 335-8505, Japan

m-nishio@tanabe.co.jp

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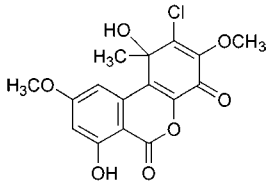
ABSTRACT



TMC-264 (1), a novel tricyclic heptaketide with a unique chloro-1*H*-dibenzo[*b,d*]pyran-4,6-dione skeleton, was discovered from the fungus *Phoma* sp. TC 1674. The structure was elucidated on the basis of NMR analyses of normal abundance and biosynthetically ^{13}C-enriched TMC-264. TMC-264 showed potent inhibitory activity against tyrosine phosphorylation of STAT6.

During the course of our screening program of microbial extracts for inhibition of IL-4 signal transduction using an IL-4 driven luciferase assay system, a novel compound, TMC-264 (**1**), was discovered from the fermentation broth of a fungus *Phoma* sp. TC 1674.[1] The structural study revealed that TMC-264 was a novel tricyclic polyketide. TMC-264 (**1**) selectively inhibited tyrosine phosphorylation of STAT6, and also inhibited the complex formation of phosphorylated STAT6 and its recognition sequence. Therefore, TMC-264 **(1)** would inhibit IL-4 signaling and would be useful in the treatment of allergic disease. We report herein the isolation and structure elucidation of TMC-264. The taxonomy, fermentation, and biological activities will be reported in a separate paper.[1]

The fermentation broth (100 flasks, 7 L) was extracted with 1-BuOH (3.5 L). The extract was concentrated and then chromatographed on a silica gel column eluted with EtOAc/*n*-hexane (1:2). Concentration of the fractions containing **1** gave a crude solid of **1** (2.1 g). The solid was purified by medium-pressure liquid chromatography on ODS developed with acetonitrile/H_2O (45:55). The relevant fractions were concentrated (1.3 g) and chromatographed on a Sephadex LH-20 column eluted with CH_2Cl_2/MeOH (1:1), yielding pure **1** (1.2 g) as pale yellow amorphous powder. Crystallization of **1** from a mixture of MeOH/H_2O afforded pale yellow needles.

The molecular formula of **1** was determined to be $C_{16}H_{13}ClO_7$ on the basis of HRESI-MS (*m/z* 351.0278 $[M - H]^-$, calcd for $C_{16}H_{12}ClO_7$ *m/z* 351.0272) in conjunction with NMR data (Table 1). The UV absorption maxima were located at λ_{max} 236 (ϵ 36 000), 266 (21 600), and 351 (9 900) nm in MeOH and acidic MeOH, and shifted to 220 (30 000), 298 (7 300), 364 (3 700), and 402 (3 800) nm in alkaline MeOH solution. **1** was soluble in $CHCl_3$, DMSO, EtOAc, MeOH, and EtOH, but insoluble in *n*-hexane and H_2O.[2]

The ^{13}C NMR spectrum of **1** displayed 16 signals composed of three methyl, two sp^2 methine, 10 quaternary sp^2, and one quaternary sp^3 carbons. The ^{1}H NMR spectrum of **1** showed two hydroxyl protons (δ 3.15 and 11.25). The

[†] Present address: Tamagawa University Research Institute, 1-1 Tamagawa Gakuen-6-chome, Machida, Tokyo 194-8610, Japan.

(1) Sakurai, M.; Nishio, M.; Yamamoto, K.; Okuda., T.; Kawano, K.; Ohnuki, T. *J. Antibiot.* Submitted for publication.

(2) ESI-MS *m/z* 353 $[M + H]^+$, 375 $[M + Na]^+$, 391 $[M + K]^+$; mp 170−171 °C; $[\alpha]^{25}_D$ −43.8° [*c* 0.5, $CHCl_3$]; IR ν_{max} [KBr] 3431, 3259, 2955, 1674, 1601, 1494, 1378, 1239, 1171, 1104, 1033, 859 cm^{-1}.

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Table 1. NMR Data of TMC-264 (**1**)

position	δ^a ^{13}C	δ^a ^{1}H (mult, *J*, Hz)	HMBC	enrichment ratios of **1** derived from [1-^{13}C]-acetate[b]	enrichment ratios of **1** derived from [2-^{13}C]-acetate[b]	enrichment ratios of **1** derived from L-[*Me*-13C]-methioninec	[1,2-$^{13}C_2$]-acetate J_{C-C} (Hz)
1-Me	29.3	1.94 (s)	1, 2, 10b	0.9	4.7	1.0	38.0
1	72.2			5.6	0.7	1.1	38.0
2	145.3			1.1	2.8	0.8	82.7
3	146.6			3.0	0.7	0.5	82.7
4	171.9			1.1	3.1	1.0	66.2
4a	141.6			3.0	0.5	0.9	66.2
6	163.6			4.8	0.7	1.0	73.6
6a	101.2			0.7	2.3	0.7	73.6
7	164.5			3.7	0.6	0.8	71.1
8	103.2	6.63 (d, 2.4)	6a, 7, 9, 10	0.9	3.1	0.6	71.1
9	166.7			4.2	0.6	0.7	67.0
10	107.0	7.61 (d, 2.4)	6, 6a, 8, 9, 10b	1.0	3.8	0.7	67.0
10a	134.1			4.1	0.6	0.8	55.4
10b	128.5			0.7	2.4	0.9	55.4
3-OMe	60.6	3.97 (s)	3	1.0	1.1	39.9	
9-OMe	56.0	3.93 (s)	9	1.0	1.0	38.7	
1-OH		3.15 (br s)					
7-OH		11.25 (s)	6a, 7, 8				

[a] Data were recorded in $CDCl_3$ at 100 and 400 MHz, respectively. [b] Enrichment ratios were normalized to peak intensities for [b]9-OMe signal. [c] Enrichment ratios were normalized to peak intensities for 1-Me signal.

connectivity of the carbons was analyzed by HMQC and HMBC data (Table 1 and Figure 1). The key HBMC

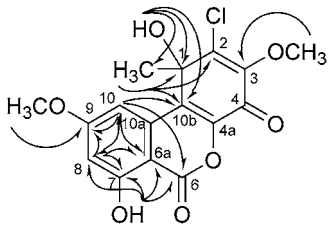

Figure 1. HMBC correlations observed in TMC-264 (**1**).

correlation from 7-OH (δ 11.25) to C-6a, 7, and 8, from H-10 (δ 7.61) to C-6a and 10b, and from 9-OMe (δ 3.93) to C-9, in conjunction with the chemical shifts and *meta*-coupling (J_{H8-H10} = 2.4 Hz), let us interpret the partial structure (position 6a−10b). Furthermore, additional HMBC correlations from 1-OH (δ 6.69) to C-1, 1-Me, and C-10b and from 7-OH to C-6 were observed when **1** was measured in DMSO-d_6.[3] Thus, the partial structure of the left half of **1** (position 6−10b−2) was established on the basis of the chemical shifts and HMBC correlations described above and NOE correlation data (9-OMe/H-8, H-10, H-10/1-Me). The remaining right half of TMC-264 (position 2−6), however, was not determined by the HMBC data.

(3) δ_C [DMSO-d_6] 70.9 (C-1), 147.2, (C-2), 145.8 (C-3), 171.9 (C-4), 141.0 (C-4a), 163.1 (C-6), 100.9, (C-6a), 163.5, (C-7), 102.7, (C-8), 165.9, (C-9), 106.5, (C-10), 134.7, (C-10a), 129.4, (C-10b), 27.8, (1-Me), 59.7, (3-Me), 56.1 (9-Me); δ_H [DMSO-d_6] 6.87 (H-8), 7.65, (H-10), 1.78, (1-Me), 3.81, (3-Me), 3.92 (9-Me), 6.69 (1-OH), 11.32 (7-OH).

To clarify the remaining carbon connectivity of **1**, incorporation experiments with [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetates and L-[*Me*-^{13}C]methionine were carried out. The ^{13}C-enriched samples of **1** were isolated from the fermentation and analyzed by ^{13}C NMR. Table 1 shows the enriched carbons in **1** after feeding of ^{13}C labeled precursors. In the experiment with [1-^{13}C]acetate, significant enrichment was observed in carbons C-1, 3, 4a, 6, 7, 9, and 10a, and with [2-^{13}C]acetate significant enrichment was observed in carbons C-2, 4, 6a, 8, 10, 10b, and 1-Me. The feeding experiment with [1,2-$^{13}C_2$]acetate followed by analysis of ^{13}C−^{13}C couplings established the incorporation pattern of intact acetate. Methyl carbons, 3-OMe and 9-OMe, were labeled by L-[*Me*-^{13}C]methionine.

As shown in Figure 2, these results demonstrated that the carbon skeleton of TMC-264 (**1**) was derived from seven acetates following the typical fungal polyketide biosynthesis

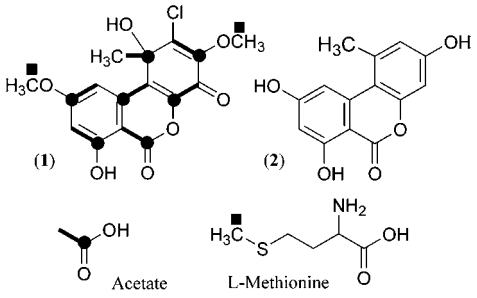

Figure 2. Distribution of label in **1** derived from [^{13}C]acetate and L-[*Me*-^{13}C]methionine and structure of alternariol (**2**).

as observed in alternariol.[4] On the basis of the large coupling constant ($^1J_{C2-C3}$ =82.7 Hz) and chemical shifts, chlorine was deduced to attach on position 2. The total structure of **1** is determined to be 2-chloro-4,6-dihydro-1,7-dihydroxy-3,9-dimethoxy-1-methyl-1*H*-dibenzo[*b*,*d*]pyran-4,6-dione.

TMC-264 (**1**) is the first compound possessing 4,6-dione or possessing 1-hydroxy group on 1-methyl-dibenzo[*b*,*d*]-pyran, although several known natural products having the same carbon skeleton have been reported like alternariol, autumnariol,[5] and graphislactone A.[6] Their carbon skeleton would be biosynthesized via a common pathway to a certain point, a single heptaketide. Judging from the fact that alternariol did not have inhibitory activity of STAT6 activation like **1**, oxidation of C-4 and introduction of chlorine to C-2 might contribute to the uniqueness of TMC-264. The stereochemistry of C-1 remains to be determined.

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Supporting Information Available: Experimental procedures and ^{1}H NMR, ^{13}C NMR, NOESY, HMQC, and HMBC spectra of normal abundance of **1**, and ^{13}C NMR of biosynthetically ^{13}C-enriched **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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