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NOTE

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Absolute Configuration of TPU-0043, a Pentaene Macrolide from *Streptomyces* sp.

Yasuhiro Igarashi, Yasuko In, Toshimasa Ishida, Tsuyoshi Fujita, Takashi Yamakawa, Hiroyasu Onaka, Tamotsu Furumai

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Abstract An antifungal pentaene macrolide TPU-0043 was isolated from *Streptomyces* sp. TP-A0625. The absolute configuration of TPU-0043 was determined to be 2*R*-(*n*-butyl)-16-methyl-3*S*,5*S*,7*S*,9*R*,11*R*,13*R*,15*S*,26*S*,27*R*-nonahydroxyoctacosa-16,18,20,22,24-pentaenoic acid, 27-lactone, by X-ray crystallography of its 13-*p*-bromobenzenesulfonyl derivative.

Keywords polyene macrolide, antifungal antibiotic, *Streptomyces*

Polyene macrolides are an important class of antifungal antibiotics and most of them are produced by actinomycetes belonging to the genus *Streptomyces*. Polyene macrolides have attracted a great deal of interest from biologists and chemists by virtue of their potent antifungal activity and structural characteristics, along with the increasing incidence and severity of acute fungal infections. A variety of synthetic methodologies have been developed to chemically characterize this unique class of natural products [1, 2]. Recently, biosynthesis of polyene macrolides has been intensively studied to generate improved fungicidal agents through genetic manipulations [3, 4]. Although more than 200 polyene macrolides have been identified, the stereochemistry of many has not been fully characterized largely because of the signal overlapping in the NMR spectra due to the iterative structural motifs and structural complexity. Among the pentaene macrolides, the absolute configuration of pentamycin (=fungichromin) [5, 6] and filipin III [2] was determined by spectral comparison of the degradation products and partial synthesis. On the other hand, the absolute configuration of rotaxaticin [7] and amphotericin B [8] was confirmed by X-ray crystallography in combination with analysis of degradation products. We herein describe the assignment of the absolute configuration of a pentaene macrolide TPU-0043.

In the screening of antifungal antibiotics from microbial secondary metabolites, *Streptomyces* sp. TP-A0625 was found to produce a polyene macrolide containing a pentaene moiety (UV λ_{max} 322, 338 and 356 nm). Strain TP-A0625 was isolated from a leaf of a perennial *Parthenocissue tricuspidata* [9] and identified as *Streptomyces* sp. on the basis of the taxonomic study. A loopful of a mature slant culture of strain TP-A0625 on Bennet's agar was inoculated into a 500-ml K-1 flask containing 100 ml of the seed medium consisting of soluble starch 1%, glucose 0.5%, NZ-case 0.3%, yeast extract 0.2%, tryptone 0.5%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, and $CaCO_3$ 0.3% (pH 7.0). The flask was incubated at 30°C for 4 days on a rotary shaker (200 rpm). Three-ml aliquots of the seed culture were transferred into one hundred 500-ml K-1 flasks each containing 100 ml of the production medium consisting of glucose 0.5%, glycerol 2%, soluble starch 2%, Pharmamedia (Trader's Protein) 1.5%, yeast extract 0.3%, KH_2PO_4 2.18%, Na_2HPO_4 1.48%, and HP-20

Y. Igarashi (Corresponding author)**, T. Yamakawa, H. Onaka, T. Furumai:** Biotechnology Research Center, Toyama Prefectural University, 5180 Kurokawa, Kosugi, Toyama 939-0398, Japan, E-mail: yas@pu-toyama.ac.jp
Y. In, T. Ishida: Department of Physical Chemistry, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1041, Japan
T. Fujita: Suntory Institute for Bioorganic Research, 1-1-1 Wakayamadai, Shimamoto, Mishima, Osaka 618-8503, Japan

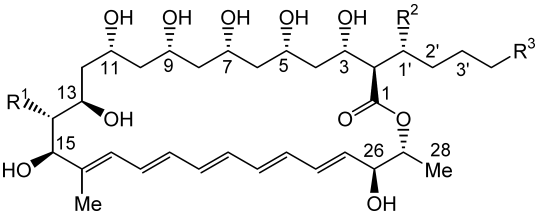
Table 1 NMR data for TPU-0043 in CD_3OD

No.	$\delta^{13}C$	$\delta^{1}H$ (integral, mult., *J* Hz)	Position
1	11.37	1.77 (3H, s)	29
2	14.24	0.90 (3H, t, 7.3)	4′
3	18.31	1.30 (3H, d, 6.1)	28
4	23.61	1.32 (2H)	3′
5	29.91	1.57 (1H), 1.73 (1H)	1′
6	30.60	1.25～1.32 (2H)	2′
7	42.48	1.38 (2H)	4, 6, 8, 10, 12, 14
8	42.67	1.72 (1H), 1.90 (1H)	
9	44.10	1.3～1.5 (2H)	
10	44.83	1.3～1.5 (2H)	
11	45.04	1.45 (1H), 1.75 (1H)	
12	45.14	1.3～1.5 (2H)	
13	54.27	2.31 (1H, ddd, 3.7, 7.6, 11.2)	2
14	67.45	3.30 (1H)	13
15	70.97	3.95～4.01 (3H)	5, 7, 26
16	73.14		
17	73.30		
18	73.39	3.81 (1H, dt, 4.6, 7.6)	3
19	73.57	3.95～4.01 (2H)	9, 11
20	74.12		
21	74.45	4.84 (1H, m)	27
22	75.62	4.14 (1H, dd, 4.2, 10.5)	15
23	128.06	6.06 (1H, d, 11.2)	17
24	129.56	6.50 (1H, dd, 11.2, 13.9)	18
25	132.37	6.28～6.42 (4H)	19～24
26	133.65		
27	134.16		
28	134.23		
29	134.44	5.91 (1H, m)	25
30	134.59	6.28～6.42 (2H)	19～24
31	134.71		
32	140.58		16
33	175.39		1

Spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Solvent peaks were used as a reference (δ_H 3.30; δ_C 49.0).

resin (Mitsubishi Chemical) 1% (pH 7). Fermentation was carried out at 30°C for 7 days on a rotary shaker (200 rpm). The fermented whole broth (10 liters) was extracted with acetone (10 liters) and the supernatant was separated from mycelium by centrifugation. The supernatant was evaporated and the resultant aqueous solution was applied on a HP-20 resin column after the pH was adjusted to 7.0. The column was eluted with 80% acetone and the eluent was evaporated and extracted with 1-butanol. The organic layer was then concentrated *in vacuo* to give a crude extract (3.65 g). It was then washed with 30% aqueous methanol and TPU-0043 (2.09 g) was obtained as a yellow crystalline solid. Recrystallization of TPU-0043 from CH_2Cl_2-MeOH afforded yellow fine needles with the melting point of 235～240°C (decomposition). The molecular formula was determined as $C_{33}H_{54}O_{10}$ by the high-resolution FAB-MS (calcd. for $[M+H]^+$ 611.3795, found 611.3789). The FT-IR spectrum (KBr) exhibited bands which implied the presence of hydroxyl groups (3375 cm^{-1}) and a lactone (1710 cm^{-1}). The UV spectrum in MeOH showed absorption maxima (log ε) at 244 (3.73), 281 (sh, 3.91), 293 (sh, 4.06), 307 (4.29), 322 (4.50), 338 (4.63), and 356 (4.62) nm, suggesting a pentaene group. Examination of the NMR and MS spectra indicated that TPU-0043 possesses the flat structure identical to chainin [10] from *Chainia* sp. and its stereoisomer, isochainin [11] from *Streptomyces cellulosae*. The structure of chainin has been determined by the MS analysis of its derivatives but the NMR and configurational assignments have not been made. The NMR data of TPU-0043 showed moderate similarity to that of isochainin but the two materials could not been conclusively distinguished. The specific rotation of chainin and isochainin is $[\alpha]_D^{25}$ −112.2° (*c* 0.16, MeOH) and $[\alpha]_D^{25}$ −24.4° (*c* 0.16, MeOH), respectively, and that of TPU-0043 was $[\alpha]_D^{23}$ −114.9° (*c* 0.2, MeOH). This good accordance in specific rotation suggests that TPU-0043 is identical with chainin; however, the authentic sample of chainin was not available and there is no definitive evidence to address their identity. Therefore, we designate the pentaene macrolide from strain TP-A0625 as TPU-0043 in this article.

To determine the absolute configuration of TPU-0043 using X-ray diffraction, we examined derivatization with a bromine-containing group and found that the reaction with *p*-bromobenzenesulfonyl chloride in pyridine at 4°C gave a desired derivative. The reaction mixture was sequentially chromatographed on silica gel and ODS column to afford a derivative (13 mg from 100 mg of TPU-0043). The ESI-TOF-MS of the derivative gave an $[M+Na]^+$ at *m/z* 851.1 (88%), 852.1 (38%), 853.1 (100%) and 854.1 (29%). This isotope peak distribution suggested the presence of a bromine atom in the molecule and thus the molecular formula was determined as $C_{39}H_{57}BrO_{12}S$. The *p*-bromobenzenesulfonate of TPU-0043 was slowly crystallized from MeOH-EtOAc to afford light yellow prisms. The prismatic crystal was analyzed by the X-ray diffraction and the absolute configuration of TPU-0043 was established as shown in Fig. 1. The *p*-bromobenzenesulfonyl group was substituted at the 13-OH group. Crystal data for 13-O-*p*-bromobenzenesulfonate of TPU-0043 at 273 K: $C_{39}H_{57}BrO_{12}S \cdot CH_3OH$, Mr=861.89, orthorhonbic, space group $P2_12_12_1$, a=9.589(1) Å, b=17.720(3) Å, c=26.227(4) Å, V=4456.5(11) $Å^3$, Z=4,



TPU-0043 (R^1=R^2=R^3=H)
Chainin, isochainin (R^1=R^2=R^3=H; configuration unknown)
Pentamycin (R^1=R^2=OH, R^3=CH_2CH_3)
Filipin III (R^1=H, R^2=OH, R^3=CH_2CH_3)

Fig. 1 Structure of TPU-0043 and related compounds.

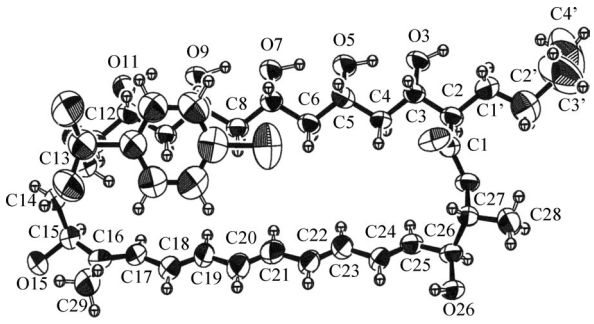


Fig. 2 Computer-generated ORTEP view drawing with atom labeling scheme and chemical drawing of 13-*p*-bromobenzenesulfonate of TPU-0043.

D_c=1.285 g cm^{-3}, μ(Mo-Kα)=0.086 mm^{-1}, F(000)=1824, χ parameter=0.014, R=0.0614. The supplementary crystallographic data (CCDC 204143) can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk). The structural differences among TPU-0043, filipin III and pentamycin lie in the length of the side chain and the number of hydroxyl groups. The side chain at C-2 of TPU-0043 is 2 carbons shorter than that of filipin III and pentamycin. Filipin III has an additional hydroxyl group at C-1′ and pentamycin has two additional hydroxyl groups at C-1′ and C-14. Except for these differences, the three pentaene macrolides possess the same absolute configuration, suggesting their relevancy in biogenesis.

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