VOL. 51, NO. 11, NOV. 1998 THE JOURNAL OF ANTIBIOTICS pp. 999 — 1003

Topostatin, a Novel Inhibitor of Topoisomerases I and II Produced by *Thermomonospora alba* Strain No. 1520

II. Physico-chemical Properties and Structure Elucidation

KEITAROU SUZUKI[a], SHOJI YAHARA[a], YUTAKA KIDO[b], KAZUHIKO NAGAO[a], YUICHI HATANO[a] and MASARU UYEDA[a,*]

Faculty of Pharmaceutical Sciences[a] and Center of Instrumental Analysis[b], Kumamoto University, 5-1 Oe-Honmachi, Kumamoto 862-0973, Japan

(Received for publication July, 15 1998)

Topostatin is a new topoisomerase inhibitor isolated from the culture filtrate of *Thermomonospora alba* strain No. 1520. The inhibitor inhibits topoisomerases I and II, and it has neither ability to stabilize the cleavable complex nor ability to intercalate into DNA strands. The molecular formula of topostatin was determined as $C_{36}H_{58}N_4O_{11}S$ based on the FAB-MS analyses, and the structure was elucidated to be a novel 14-membered ring containing peptide and terpenoid by various NMR spectroscopies.

We have isolated a potent topoisomerases inhibitor, topostatin (Fig. 1), from the culture filtrate of *Thermomonospora alba* strain No. 1520. Topostatin showed the inhibitory activities (IC_{50}) against topoisomerase I (13 ng/μl) and topoisomerase II (3 ng/μl) without the stabilization of cleavable complex and DNA intercalation. In the preceding paper[1], the taxonomy of producing organism, fermentation, purification procedure and biological activities of topostatin were reported. In this paper, we describe the physico-chemical properties and structure elucidation of topostatin.

Results

Physico-chemical Properties

Physico-chemical properties of topostatin are summarized in Table 1. Topostatin was obtained as a yellowish white powder. The inhibitor was soluble in pyridine and partially soluble in water, dimethylsulfoxide, methanol, ethanol and insoluble in acetone, ethylacetate, chloroform and ether. The melting point and optical rotation were found 179~186°C (decomposition) and +18.3°, respectively. The UV spectrum of topostatin exhibited maximum absorption at 285 nm ($\varepsilon = 2.66 \times 10^4$) in

Fig. 1. Structure of topostatin.

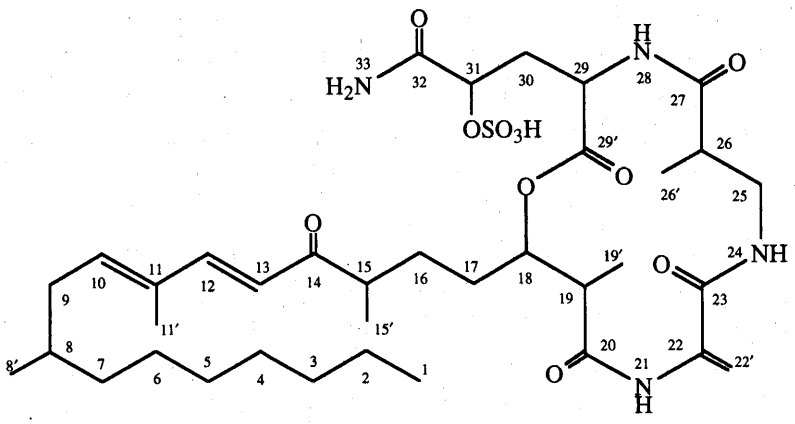


Table 1. Physico-chemical properties of topostatin.

Appearance	: yellowish white powder
MP (°C)	: 179 ~ 186 (decomposition)
$[\alpha]_D^{28}$ (c = 0.1, MeOH)	: +18.3°
UV λ max (MeOH) nm (ε)	: 285 (2.66×10^4)
IR ν_{max}^{KBr} cm^{-1}	: 3458, 3309, 1730, 1672, 1628, 1259
TLC (Rf) [a]	: 0.27
HPLC (Rt) [b]	: 6.40
Solubility	:
Soluble	pyridine
Partially soluble	H_2O, DMSO, MeOH, EtOH
Insoluble	acetone, AcOEt, $CHCl_3$, ether
Formula	: $C_{36}H_{58}N_4O_{11}S$
HRFAB-MS	: $(M-H+2Na)^+$
Found	799.3538
Calcd	799.3540

a) Silica gel 60 F_{254}, $CHCl_3$-EtOH (7:3).
b) LiChroCART Lichrosorb RP-Select B (7 μm), mobile phase; MeOH-H_2O (7:3), flow rate; 0.8 ml/min, detection; 285 nm.

methanol. Topostatin showed a spot with Rf value of 0.27 on Silica gel 60 F_{254} sheet using solvent system of chloroform-ethanol (7:3). Coinciding with a single peak, HPLC on LiChroCART Lichrosorb RP-Select B with methanol-water (7:3) as mobile phase, resulted in 6.4 minutes of retention time. Strong absorption at 3458, 3309, 1730, 1672, 1628 and 1259 cm^{-1} in IR spectrum suggested the presence of amide, ester and ketone. Based on HRFAB-MS experiment, the molecular weight of topostatin was determined to be 754 and its molecular formula was established as $C_{36}H_{58}N_4O_{11}S$ [*m/z* 799.3538 $(M-H+2Na)^+$, Calcd 799.3540].

Structure Elucidation

Topostatin showed a $[M-H+2Na]^+$ parent peak at *m/z* 799 and a fragment ion peak at *m/z* 697 lost the SO_3Na ion from the parent peak at *m/z* 799 in the positive FAB-MS. In ^1H NMR and ^1H-^1H COSY spectra of topostatin (Table 2), the proton signals at δ 0.84 (CH_3-8′), 0.85 (CH_3-1), 1.14 (CH_3-15′), 1.21 (CH_3-26′), 1.31 (CH_3-19′) and 1.85 (CH_3-11′) were assigned to the methyl groups, and also the proton signals at δ 6.00 (CH-10), 5.44 (CH_2-22′), 6.11 (CH_2-22′), 6.54 (CH-13) and 7.54 (CH-12) were assigned to the olefinic groups. The proton signals at δ 8.18 (NH_2-33, coupling with δ 8.33), 8.22 (NH-24, coupling with δ 3.27 and δ 4.18 (CH_2-25)), 8.33 (NH_2-33, coupling with δ 8.18), 9.36 (NH-28) and 10.49 (NH-21) were assigned to the amide groups, because these signals were exchanged by the measurement in D_2O. The ^{13}C NMR (Table 2) spectrum of topostatin exhibited 36 carbon signals, and these signals attributed to 6 methyl signals, 1 vinyl group (δ 114.6 and 139.2), 2 double bonds (δ 123.3, 133.7, 143.7 and 147.8), 1 carbonyl (δ 203.7), 5 amide and ester carbonyls (δ 165.5, 172.3, 173.8, 174.8 and 175.1), 2 oxygenated methines (δ 75.1 and 76.9), 5 methines and 11 methylenes, therefore, topostatin is a terpenoid-peptide derivative. Normal amino acids were not detected in the acid hydrolysate of topostatin (6N HCl, 115°C, 24 hours, data not shown). Full assignments of the ^1H and ^{13}C signals were secured by the ^1H-^1H COSY, HMQC and HMBC spectra (Table 2).

In the HMBC spectrum (8 Hz) of topostatin, the proton signals of topostatin showed $^2J_{C-H}$, $^3J_{C-H}$ and $^4J_{C-H}$ correlations with the carbon signals (Table 2). The proton signals at δ 0.85 (CH_3-1), 0.84 (CH_3-8′), 1.85 (CH_3-11′), 1.14 (CH_3-15′), 1.31 (CH_3-19′) and 1.21 (CH_3-26′) correlated with the carbon signals at δ 22.9 (CH_2-2), 32.1 (CH_2-3) and 29.9 (CH_2-4); δ 37.1 (CH_2-6), 36.4 (CH_2-7) and 32.7 (CH-8); δ 143.7 (CH-10), 133.7 (C-11) and 147.8 (CH-12); δ 203.7 (CO-14), 44.3 (CH-15) and 28.1 (CH_2-16); δ 76.9 (CH-18) and 44.5 (CH-19); and δ 173.8 (CO-20), 42.6 (CH-25), 41.7 (CH-26) and 175.1 (CO-27), respectively. Moreover, the proton signals at δ 2.13 (CH_2-9), 6.54 (CH-13), 5.51 (CH-18), 2.98

Table 2. ^{13}C NMR and ^{1}H NMR assignments and ^{1}H-^{13}C long-range correlations of topostatin by ^{1}H-^{1}H COSY, HMQS and HMBC in pyridine-d_5.

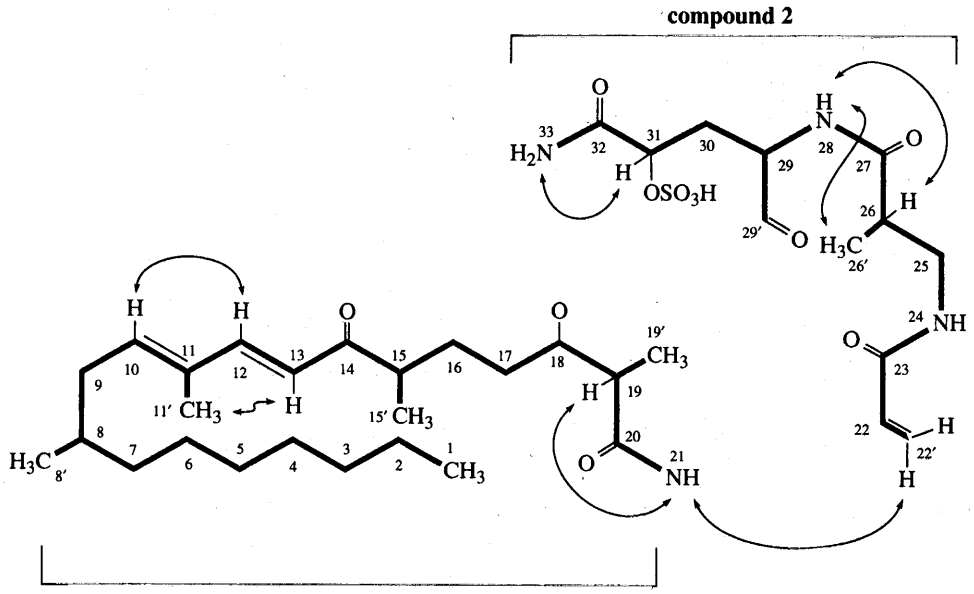
Position	δ_C		δ_H	Cross peaks (δ_C) in HMBC spectrum
1	14.3	CH_3	0.85 (t, 6.7)	22.9 (2), 29.9 (4), 32.1 (3)
2	22.9	CH_2	1.22 (m)	
3	32.1	CH_2	1.22 (m)	
4	29.9	CH_2	1.22 (m)	
5	27.1	CH_2	1.22 (m), 1.27 (m)	
6	37.1	CH_2	1.22 (m), 1.96 (m)	
7	36.4	CH_2	1.22 (m)	
			1.37 (m)	19.6 (8′), 27.1 (5), 37.1 (6)
8	32.7	CH	1.35 (m)	19.6 (8′), 26.8 (9), 36.4 (7)
8′	19.6	CH_3	0.84 (d, 6.1)	32.7 (8), 36.4 (7), 37.1 (6)
9	26.8	CH_2	2.13 (m)	32.7 (8), 36.4 (7), 133.7 (11), 143.7 (10)
10	143.7	CH	6.00 (t, 7.3)	12.4 (11′), 26.8 (9), 36.4 (7), 147.8 (12)
11	133.7	C		
11′	12.4	CH_3	1.85 (s)	133.7 (11), 143.7 (10), 147.8 (12)
12	147.8	CH	7.54 (d, 15.8)	12.4 (11′), 133.7 (11), 143.7 (10), 203.7 (14)
13	123.3	CH	6.54 (d, 15.8)	133.7 (11), 203.7 (14)
14	203.7	CO		
15	44.3	CH	2.96 (m)	16.7 (15′), 28.1 (16), 203.7 (14)
15′	16.7	CH_3	1.14 (d, 6.7)	28.1 (16), 44.3 (15), 203.7 (14)
16	28.1	CH_2	1.66 (m),	203.7 (14)
			2.01 (m)	
17	30.1	CH_2	1.82 (m), 2.03 (m)	
18	76.9	CH	5.51 (m)	28.1 (16), 44.8 (19), 173.8 (20)
19	44.8	CH	2.96 (m)	15.2 (19′), 28.1 (16), 30.1 (17), 76.9 (18), 173.8 (20)
19′	15.2	CH_3	1.31 (d, 7.3)	44.8 (19), 76.9 (18), 173.8 (20)
20	173.8	CO		
21		NH	10.49 (br.s)	173.8 (20)
22	139.2	C		
22′	114.6	CH_2	5.44 (br.s),	139.2 (22), 165.5 (23)
			6.11 (br.s)	165.5 (23)
23	165.5	CO		
24		NH	8.22 (br.d, 6.0)	
25	42.6	CH_2	3.27 (br.d, 13.4),	41.7 (26), 165.5 (23), 175.1 (27)
			4.18 (m)	41.7 (26), 165.5 (23), 175.1 (27)
26	41.7	CH	2.88 (m)	14.8 (26′), 42.6 (25), 175.1 (27)
26′	14.8	CH_3	1.21 (d, 6.7)	41.7 (26), 42.6 (25), 175.1 (27)
27	175.1	CO		
28		NH	9.36 (br.d, 7.5)	175.1 (27)
29	50.1	CH	5.40 (m)	172.3 (29′), 175.1 (27)
29′	172.3	CO		
30	34.3	CH_2	2.78 (br.t, 10.0),	50.1 (29)
			3.33 (m)	
31	75.1	CH	5.63 (dd, 3.7, 10.3)	34.3 (30), 50.1 (29), 174.8 (32)
32	174.8	CO		
33		NH_2	8.18 (br.s),	
			8.33 (br.s)	75.1 (31)

(CH-19), 5.40 (CH-29) and 5.63 (CH-31) showed correlations with the carbon signals at δ 36.4 (CH_2-7), 32.7 (CH-8), 143.7 (CH-10) and 133.7 (C-11); δ 133.7 (C-11) and 203.7 (CO-14); δ 28.1 (CH_2-16), 44.5 (CH-19) and 173.8 (CO-20); δ 30.1 (CH_2-17), 76.9 (CH-18), 15.2 (CH_3-19′) and 173.8 (CO-20); δ 175.1 (CO-27) and 172.3 (CO-29′); and δ 50.1 (CH-29), 34.3 (CH-30) and 174.8 (CO-32), respectively. The proton signals of NH at δ 10.49 (NH-21), 9.36 (NH-28) and 8.33 (NH_2-33) correlated with the carbon signals at δ 173.8 (CO-20), 175.1 (CO-27) and 75.1 (CH-31), respectively. From the above evidences, the two main skeletons which are compound **1** consisting of CH_3-1 to NH-21 and compound **2** consisting of CH_2-22′ to NH_2-33 in the structure of topostatin were elucidated as shown in Fig. 2.

The linkage of NH-21 to C-22 was showed by correlations with the proton signals at δ 10.49 (NH-21) and 5.44 (CH_2-22′) in the NOESY spectrum (Fig. 2). Thus, the correlations of all carbons and protons were recognized. The positions of ester and sulfate were

Fig. 2. Correlations observed for topostatin by HMBC and NOESY.

——; ^{1}H-^{13}C long range correlations by HMBC.
←→; nuclear overhauser effects by NOESY.



analyzed by IR spectrum. The absorption at 1730 cm^{-1} suggested that the ester was normal ester bond. Therefore, the ester should be located at C-18 oxygen to C-29′ carbonyl, and the sulfate linked at C-31 oxygen position. From these results, the structure of topostatin was elucidated as shown in Fig. 1. And also, it was suggested from the correlations of the proton signals at δ 6.00 (CH-10) and 7.54 (CH-12), and δ 1.85 (CH_3-11′) and 6.54 (CH-13) in NOESY spectrum that the Δ^{10} and Δ^{12} double bonds were *E* conformation.

Discussion

The molecular formula of topostatin was determined as $C_{36}H_{58}N_4O_{11}S$ based on MS analyses, and its structure was established by spectroscopic analyses, mainly by NMR techniques. Topostatin is a novel 14-membered ring containing terpenoid and peptide which consists of uncommon amino acids.

The known topoisomerase inhibitors such as camptothecin[2] and doxorubicin[3] inhibit topoisomerases by stabilizing the cleavable complexes[4]. And some inhibitors such as diketopiperazine family [5~7] and CJ-12373[8] inhibit topoisomerase by direct action on the enzyme molecule without stabilizing the complex. However, these inhibitors have no ability to inhibit both the enzymes, topoisomerases I and II. On the other hand, topostatin can inhibit both the enzymes without stabilizing the cleavable complexes. Topostatin maybe useful tools for cancer therapy in the future. The stereochemistry and total synthesis of topostatin are now under investigation, and the results will be described in our next papers.

Experimental

^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HMQC and heteronuclear multiple bond correlation (HMBC) spectra were measured by a JNM α-500 spectrometer. NMR spectra with tetramethylsilane (TMS) as an internal standard were taken in pyridine-d_5 solution at 500 MHz (^{1}H) and 125 MHz (^{13}C). FAB-MS spectra were recorded with a JMS-DX 303 HF and JMS-HX 100 spectrometers. Optical rotation, UV spectrum and IR spectrum were measured by a Jasco DIP-1000KUY digital polarimeter, Hitachi U-2000 spectrometer and Hitachi 270-30 spectrophotometer, respectively.

Acknowledgments

We are grateful to Dr. F. ABE and Mr. H. HANAZONO of Fukuoka University, and Dr. Y. ITAGAKI of Suntory Institute for Bioorganic Research for MS measurements.

References

1) SUZUKI, K.; K. NAGAO, Y. MONNAI, A. YAGI & M. UYEDA: Topostatin, a novel inhibitor of topoisomerases I and II produced by *Thermomonospora alba* strain No. 1520. I. Taxonomy, fermentation, isolation and biological activities. J. Antibiotics 51: 991~998, 1998
2) HSIANG, Y. H.; R. HERTZBERG, S. HECHT & L. F. LUI: Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J. Biol. Chem. 260: 14873~14878, 1985
3) TEWEY, K. M.; T. C. ROWE, L. YANG, B. D. HALLIGAN & L. F. LIU: Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. Science 226: 466~468, 1984
4) SCHNEIDER, E.; Y.-H. HSIANG & L. F. LIU: DNA topoisomerases as anticancer drug targets. Adv. Pharmacol. 21: 149~183, 1990
5) FUNABASHI, Y.; T. HORIGUCHI, S. IINUMA, S. TANIDA & S. HARADA: TAN-1496 A, C and E, diketopiperazine antibiotics with inhibitory activity against mammalian DNA topoisomerase I. J. Antibiotics 47: 202~1218, 1994
6) TANABE, K.; Y. IKEGAMI, R. ISHIDA & T. ANDOH: Inhibition of topoisomerase II by antitumor agents bis (2,6-dioxopiperazine) derivatives. Cancer Res. 51: 4903~4908, 1991
7) ISHIDA, R.; T. MIKI, T. NARITA, R. YUI, M. SATO, K. R. UTSUMI, K. TANABE & T. ANDOH: Inhibition of intracellular topoisomerase II by antitumor bis (2,6-dioxopiperazine) derivatives: mode of cell growth inhibition distinct from that of cleavable complex-forming type inhibitors. Cancer Res. 51: 4909~4916, 1991
8) INAGAKI, T.; K. KANEDA, Y. SUZUKI, H. HIRAI, R. NOMURA, T. SAKAKIBARA, Y. YAMAUCHI, L. HUANG, M. NORCIA, L. M. WONDRACK, J. A. SUTCLIFFE & N. KOJIMA: CJ-12373, a novel topoisomerase II inhibitor: Fermentation, isolation, structure elucidation and biological activities. J. Antibiotics 51: 112~116, 1998