NOTES

41-Demethylhomooligomycin B, a New Immunosuppresant Antibiotic from *Streptomyces ostreogriseus*

HANG SUB KIM, SANG BAE HAN, HWAN MOOK KIM, YOUNG HO KIM and JUNG JOON LEE*

Korea Research Institute of Bioscience and Biotechnology, KIST, P.O. Box 115, Yusung, Taejon 305-600, Korea

(Received for publication July 15, 1996)

In screening for new immunosuppresive agents, we isolated a novel oligomycin analogue, 41-demethylhomooligomycin B (**1**) from the culture broth of *Streptomyces* sp. MCH79 which was isolated from soil collected in Yusung, Korea. This strain was identified as *Streptomyces ostreogriseus* on the basis of its cultural, morphological and physiological properties.

A slant culture of the strain MCH79 was used to inoculate 100 ml of SP medium consisting of soluble starch 2%, glucose 1%, soybean meal 2.5%, beef extract 0.1%, yeast extract 0.4%, NaCl 0.2%, $CaCO_3$ 0.2% and K_2HPO_4 0.005% (pH 7.2) in a 500-ml baffled flask. The flask was shaken on a rotary shaker for 3 days at 28°C. Five liters of seed broth was transferred to a 300-liter fermenter (Korea Fermenter Co., Ltd.) containing 150 liter of SP medium and was cultivated for 3 days at 28°C, 300 rpm and aeration of 110 liters/minute.

The mycelial cake, separated from the fermentation broth by centrifugation, was extracted with acetone and the aqueous acetone filtrate was extracted twice with ethyl acetate. The yellow oily residue obtained after evaporation of organic solvent *in vacuo* was applied on a Sephadex LH-20 column and eluted with methanol. The active fractions were concentrated and chromatographed on a MPLC column (RP-18, 26 × 460 mm, 60 Å, 20~45 μ, EUROCHROM; mobile phase 75% MeOH; flow rate 23 ml/minute; detection, UV 220 nm). Finally, **1** was obtained as a white powder (46 mg) by HPLC (column, ODS, 19 × 300 mm, Deltapak; mobile phase 65% MeOH; flow rate 40 ml/minute; detection UV 232 nm). The physico-chemical properties of **1** are summarized in Table 1. The molecular formula was determined as $C_{45}H_{72}O_{12}$ by HRFAB-MS ($M+Na^+$, m/z, calcd: 827.4921, found: 827.5004). The IR spectra (Laser Precision Analitical, IFX-65s) indicated the existence of hydroxyl (3465 cm^{-1}) and carbonyl (1705 cm^{-1}) groups. The presence of a conjugated diene system was also indicated by the IR (1641 cm^{-1}) and UV (λ_{max}^{MeOH} 225 nm) spectra. These spectral properties suggested **1** to be an oligomycin.[1] The ^1H and ^{13}C NMR spectral data are shown in Table 2. The ^{13}C NMR data indicated that **1** contained three double bonds in a conjugated diene, and four carbonyl groups, which meant that **1** might belong to the oligomycin B group having a C-28 carbonyl moiety.[2] The ^1H-^1H COSY, ^{13}C-^1H COSY, and HMBC experiments indicated that **1** contained a 26 membered α,β-unsaturated lactone with a conjugated diene fused to a bicyclic spiroketal moiety and one ethyl group.[3] The molecular forumula of **1** was the same as that of oligomycin B, but the chemical shifts of proton/carbon and coupling patterns of protons around C-20 and C-26 were much different from those of oligomycin B. The HMBC correlations of the C-41 with H-19, H-20 and H-21, and C-20 with the methyl protons of C-41 gave important information regarding the substitution of methyl for ethyl residue at the C-20 position of oligomycin B. Another HMBC correlation of C-44' with H-26 and H-44 meant that **1** should have an ethyl group at C-26. These data suggested that the structure of **1** was 41-demethylhomooligomycin B. Homologues of oligomycin having an ethyl group at C-26 were already reported as homooligomycin A and B,[4] but the analogues of demethylated oligomycin at C-41 are first described in this report.

Among oligomycin analogues, oligomycin F and A are known to have immunosuppressive activities.[5] These derivatives suppress the immune functions such as mixed

Fig. 1. Structure of 41-demethylhomooligomycin B.

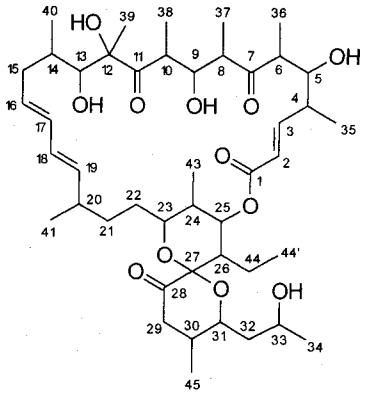


Table 1. Physico-chemical properties of **1**.

Appearance	White powder
MP (°C)	118~120
$[\alpha]_D^{25}$ (c 1.37, CH_3OH)	−54°
Molecular formula	$C_{45}H_{72}O_{12}$
HRFAB-MS (m/z)	
Calcd:	827.4921
Found:	827.5004 $(M+Na)^+$
UV λ_{max}^{MeOH} nm (ε)	225 (47,873), 232 (44,698), 241 (27,112)
IR (KBr) cm^{-1}	3465, 2968, 1705 1641, 1460, 1381, 989
Rf value[a]	0.36

[a] Silica gel TLC (Merck Art. 5715), solvent; $CHCl_3$-CH_3OH (9:1).

Table 2. ^{1}H and ^{13}C NMR data of **1**.

Position	$\delta^{13}C^{a}$ (*J*, Hz)		$\delta^{1}H^{b}$ (*J*, Hz)
1	165.01	C=O	—
2	122.52	CH	5.85 (1H, d, *J*=15.6 Hz)
3	149.11	CH	6.68 (1H, dd, *J*=15.6, 10.0 Hz)
4	40.20	CH	2.41 (1H, m)
5	72.99	CH	3.79 (1H, d, *J*=9.9 Hz)
6	46.66	CH	2.75 (1H, m)
7	220.09	C=O	—
8	45.90	CH	2.77 (1H, m)
9	72.61	CH	3.99 (1H, m)
10	41.81	CH	3.63 (1H, m)
11	220.19	C=O	—
12	83.05	C	—
13	71.98	CH	3.94 (1H, d, *J*=4.4 Hz)
14	33.60	CH	1.87 (1H, m)
15	38.36	CH_2	2.22 (1H, m), 2.03 (1H, m)
16	129.27	CH	5.93 (1H, m)
17	132.12	CH	6.03 (1H, m)
18	130.05	CH	5.46 (1H, ddd, *J*=14.7, 10.6, 4.0 Hz)
19	138.36	CH	5.26 (1H, dd, *J*=14.7, 9.4 Hz)
20	38.41	CH	2.06 (1H, m)
21	32.96	CH_2	2.90 (1H, m), 1.68 (1H, m)
22	30.66	CH_2	1.55 (1H, m), 1.50 (1H, m)
23	70.83	CH	4.03 (1H, m)
24	35.93	CH	2.15 (1H, m)
25	75.97	CH	5.12 (1H, dd, *J*=11.8, 4.8 Hz)
26	36.77	CH	2.40 (1H, m)
27	101.11	C	—
28	202.97	C=O	—
29	43.91	CH_2	3.04 (1H, m), 2.16 (1H, m)
30	36.77	CH	2.20 (1H, m)
31	67.06	CH	4.52 (1H, d, *J*=10.3 Hz)
32	41.69	CH_2	1.63 (1H, m), 1.41 (1H, m)
33	64.58	CH	4.08 (1H, m)
34	25.01	CH_3	1.28 (3H, d, *J*=6.2 Hz)
35	17.75	CH_3	1.18 (3H, d, *J*=6.5 Hz)
36	8.40	CH_3	1.08 (3H, d, *J*=7.3 Hz)
37	9.47	CH_3	1.05 (3H, d, *J*=6.9 Hz)
38	13.91	CH_3	1.11 (3H, d, *J*=6.9 Hz)
39	20.99	CH_3	1.13 (3H, s)
40	14.48	CH_3	1.01 (3H, d, *J*=6.6 Hz)
41	21.53	CH_3	1.02 (3H, d, *J*=6.6 Hz)
42	—	—	
43	5.92	CH_3	0.85 (3H, d, *J*=6.9 Hz)
44	21.85	CH_2	1.42 (1H, m), 1.31 (1H, m)
44′	13.12	CH_3	0.92 (3H, t, *J*=7.5 Hz)
45	13.05	CH_3	0.98 (3H, d, *J*=6.9 Hz)

[a] $CDCl_3$, 125.77 MHz; [b] $CDCl_3$, 500.14 MHz.

Fig. 2. ^{1}H-^{13}C long range coupling observed in HMBC spectrum of 41-demethylhomooligomycin B (arrows are directed from H to C).

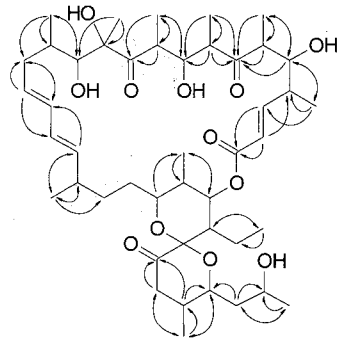
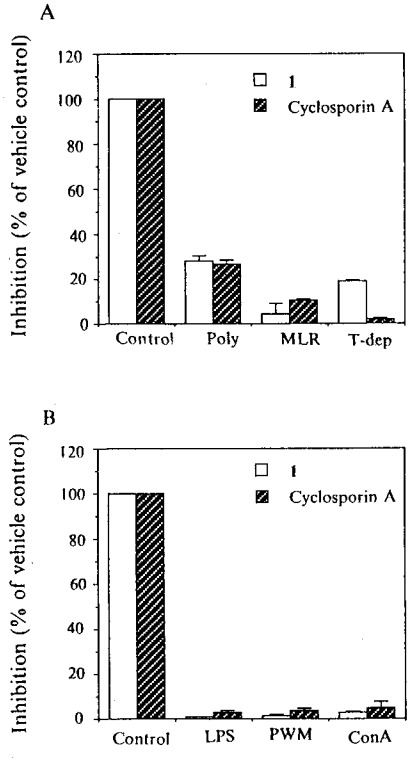


Fig. 3. Immunosuppressive activities of **1** on the functions of mouse splenocytes.



Mouse spleens were used to prepare lymphocytes. Immune functions of lymphocytes were tested in the presence of 1 μg/ml of **1** or cyclosporin A. The data was expressed as a mean±standard deviation of six separate determinations. Results were calculated as percent inhibition to vehicle control. A; The polyclonal B cell activation (poly), T cell activation (MLR) and T-dependent antibody response (T-dep), B; The blastogenesis by LPS, PWM and ConA.

lymphocyte reaction (MLR), blastogenesis by pokeweed mitogen (PWM) and IgG production. In the present study, 41-demethylhomooligomycin B (**1**), a novel analogue of oligomycin, was evaluated for the immunosuppressive activities. Various immune functions such as primary T-dependent IgM response, B cell activation and T cell activation have been determined (Fig. 3). Mouse (female BDF1, 20~25 g of body weight) spleens were used to prepare lymphocytes. Primary IgM response was induced by the incubation of lymphocytes (10^{7} cells/ml) with sheep red blood cells (1.3×10^{7} cells/ml) for 5 days.[6] B cell activation was made by the addition of LPS (25 mg/ml) and further incubation for 2 days.[6] To cause T cell activation, splenic lymphocytes were mixed with splenocytes of female B6C3F1 and incubated for 3 days.[7] The blastogenesis induced by LPS, PWM, and concanavalin A (ConA) was also examined. The proliferation of lymphocytes was induced by incubation with 5 mg/ml

of each mitogen for 3 days.[8] The antibody secretions were determined by suspension hemolytic assay[6] and lymphocyte proliferations was monitored by ^{3}H-thymidine uptake.[8] The results showed that **1** exhibited 71.7% suppression of B cell activation, 95.5% of T cell activation and 80.8% of T-dependent IgM response at a final concentration of 1 μg/ml. The induced proliferation of T and B cells by mitogens (LPS, PWM, ConA) were also fully suppressed at the same concentration of **1**. The suppressive potency of **1** was comparable to that of cyclosporin A which was used as a positive control. In conclusion, these results indicate that **1** may be a potent and broad suppressive agent on the functions of mouse splenic lymphocytes.

Acknowledgments

This research was partially supported by a grant from the Korea Ministry of Science and Technology in Korea.

References

1) PROUTY, W. F.; R. M. THOMPSON, H. K. SCHNOES & F. M. STRONG: Oligomycin: Degradation products and part structure of oligomycin B. Biochem.Biophy. Res. Comm. 44: 619~627, 1971
2) CARTER, G. T.: Structure determination of oligomycin A and C. J. Org. Chem. 51: 4264~4271, 1986
3) SZILAGYI, L.; J. SAMU & I. HARSANYI: Structure elucidation of two acetylated derivatives of oligomycin A. Spectroscopy Lett. 28: 699~707, 1995
4) YAMAZAKI, M.; T. YAMASHITA, T. HARADA, T. NISHIKIORI, S. SAITO, N. SHIMADA & A. FUJII: 44-Homooligomycins A and B, new antitumor antibiotics from *Streptomyces bottropensis*: Producing organism, fermentation, isolation, structure elucidation and biological properties. J. Antibiotics 45: 171~179, 1992
5) LAATSCH, H.; M. KELLNER, G. WOLF, Y.-S. LEE, F. HANSSKE, S. KONETSCHNY-RAPP, U. PESSARA, W. SCHEUER & H. STOCKINGER: Oligomycin F, a new immunosuppressive homologue of oligomycin A. J. Antibiotics 46: 1334~1341, 1993
6) CROWLEY, M. T.; K. INABA, M. D. WITMER-PACK, S. GEZELTER & R. M. STEINMAN: Use of the fluorescence activated cell sorter to enrich dendritic cells from mouse spleen. J. Immunol. Methods 133: 55~66, 1990
7) KIM, H. M.; S. B. HAN, W. I. CHANG, B. H. HYUN, G. T. OH, C. J. AHN & Y. N. CHA: Selective suppression of *in vitro* T-dependent humoral immunity by synthetic food additive antioxidants. J. Toxicol. Sciences 21: 41~45, 1996
8) WOOD, S. C.; J. G. KARRAS & M. P. HOLSAPPLE: Integration of the human lymphocyte into immunotoxicological investigations. Fundamental and Applied Toxicology 18: 450~459, 1992