Journal of Microbiology and Biotechnology
Vol. 7. No. 4, 272-277, 1997

Immunosuppressive Activity of Elaiophylins

LEE, SANG-YONG[1], HANG-SUB KIM, YOUNG-HO KIM[†], SANG-BAE HAN, HWAN-MOOK KIM, SOON-DUCK HONG[1], AND JUNG-JOON LEE*

Natural Products Biosynthesis R.U., Korea Research Institute of Bioscience and Biotechnology (KRIBB), P.O. Box 115, Yusong, Taejon 305-600, Korea
[1]Department of Microbiology, Kyungpook National University, Taegu 702-701, Korea

In the purification of elaiophylin from a culture of *Streptomyces hygroscopicus* MCY-846, mono- and dimethyl-elaiophylin were obtained through a *O*-methylation of the hemiketal hydroxy group of elaiophylin. All the three elaiophylins showed cytotoxicity against several human tumor cell lines and murine cell lines. Elaiophvlin and monomethyl-elaiophylin also showed antimicrobial activities against gram-positive bacteria and potent inhibitory effects on the activation of B cells by lipopolysaccharide as well as on the proliferation of mouse splenic lymphocytes stimulated by mitogens but dimethyl-elaiophylin did not. This result indicats that elaiophylin and monomethyl-elaiophylin would be strong immunosuppressants. Furthermore this result revealed an interesting structure-activity relationship suggesting that the lack of symmetry and /or the free OH group at C-11 of elaiophylin might be important in conferring biological activities.

The immune system evolved to defend vertebrates against infection and composed of various cells with distinct functions. Highly selective immunomodulating substances are expected not only to be useful tools for the study of cellular and biochemical events of immune responses, but also potent clinical use for immune diseases. In our continuing effort to search for novel antitumor or immunosuppressive compounds from microbial metabolites, elaiophylin was isolated from *Streptomyces hygroscopicus* MCY-846 (15). During the purification of elaiophylin, two elaiophylins, monomethyl- and dimethyl-elaiophylin, were isolated and it was turned out that they were formed by *O*-methylation of the hemiketal hydroxy group of elaiophylin by a solvent system containing methanol. Elaiophylin and monomethyl-elaiophylin were identified as potent immunosuppressive agents but dimethyl-elaiophylin was not.

Elaiophylin is a macrolide antibiotic with 16-membered unsaturated lacton ring like leucanicidine (7), hygrolidine (22), bafilomycins (25), avermectins (3), and was initially isolated from the cultures of *Streptomyces melanosporus* (1) and *Streptomyces violaceoniger* (4). Due to the characteristic C2 symmetry structure there were several reports on the synthesis and structure-activity relationships of elaiophylin derivatives (6, 24) but no report on the physico-chemical properties of monomethyl-elaiophylin including ^{1}H- and ^{13}C-NMR study and the immunosuppressive activity of elaiophylin and monomethyl-elaiophylin. We described the ^{1}H- and ^{13}C-NMR data of monomethyl-elaiophylin and the immunosuppressive activity of monomethyl-elaiophylin and elaiophylin in this report.

*Corresponding author
[†]Present address: College of Pharmacy, Chungnam National University, Taejon 305-764, Korea
Phone: 82-42-860-4360. Fax: 82-42-860-4595.
E-mail: jjlee@kribb4680.kribb.re.kr
Key words: *Streptomyces hygroscopicus* MCY846, elaiophylin, immunosupressive

MATERIAL AND METHODS

Matreials

SPF *Balb/c*-KIST mice weighing 17-20 g were supplied by the Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea. The animals were kept in a clean room according to the usual maintenance procedures. RPMI1640 was purchased from GIBCO BRL, Grand Island, NY, U.S.A. Fetal Calf serum (FCS) was supplied by HyClone Lab., UT, U.S.A. Sheep red blood cell (SRBC), suspended in alsever's solution, were obtained from Korea Media Ltd., Seoul, Korea. Lipopolysaccharide (LPS), and the other chemicals were purchased from Sigma Co., U.S.A.

Cells and Culture Conditions

The strain was isolated from a soil sample collected in Cheju-Do, Korea (15). Based on taxonomic properties such as cultural characteristics on ISP media, chemotaxonomic data, utilization of carbon and nitrogen

sources, the isolate MCY-846 was identified as a *Streptomyces hygroscopicus* (17). A slant culture of the strain MCY-846 grown on modified Bennett's agar medium (1% glucose, 0.2% bacto-peptone, 0.1% beef extract, 0.1% yeast extract, pH 7.2) was inoculated into 500 ml baffled flasks containing 100 ml of seed medium (2% soluble starch, 1% glucose, 0.1% beef extract, 0.4% yeast extract, 0.2% NaCl, 0.005% K_2HPO_4, 0.2% $CaCO_3$, 2.5% soybean meal). The flasks were shaken on a rotary shaker for 3 days at 28°C and 200 rpm. The seed cultures (200 ml) were transfered into 5 liter medium in a 7.5-liter jar fermentor. Fermentation was carried out for four days at 28°C, 200 rpm and with aeration of 200 liters/h.

Isolation and Purification

The culture broth (10 liters) of *S. hygroscopicus* MCY-846 which was grown in a GSS medium was separated into supernatant and mycelium by centrifugation. The mycelium was extracted with acetone and the solvent was evaporated *in vacuo*. The aqueous residue was extracted with CH_2Cl_2. The concentrated CH_2Cl_2 extract was fractionated on a MPLC (column, RP-18, ϕ 26×460 mm, 60Å, 20~45 μm, EUROCHROM®; mobile phase 83% MeOH; flow rate 25 ml/min; detection UV 253 nm). The cytotoxic fractions were further purified by preparative HPLC (column, ODS, ϕ 19×300 mm, Delta Pak, mobile phase 82% MeOH, flow rate 40 ml/min, detection UV 253 nm) to give dimethylelaiophylin (42 mg), monomethylelaiophylin (47 mg), and elaiophylin (25 mg) at a retention time of 83 min, 26 min, 20 min, respectively.

Analytical Methods

The ^1H and ^{13}C-NMR spectra were recorded on a Varian UNITY 300 NMR spectrophotometer using standard pulse sequences. Chemical shifts are reported in δ value referenced to $CDCl_3$ (δ 7.26) for ^1H and $CDCl_3$ (δ 77) for ^{13}C as internal reference and coupling constant (J) are given in Hz. Electrospray Interface (ESI) mass spectral data were obtained on a JMS-HS 110A spectrometer. The UV absorption spectra were measured with a Milton Roy spectronic 3000 array spectrophotometer. The IR absorption spectra were obtained with KBr disks on a Laser Precision Analytical RFX-65 FT-IR. The melting points were determined on a model of Electrothermal 9100 without correction. Optical rotations were determined on a JASCO DIP-181 polarimeter. Preparative HPLC was carried out on a DELTA-PAK C18 (ϕ 19×300 mm, Waters) and monitored with a UV detector at 253 nm.

***In vitro* Cytotoxicity and Antimicrobial Activity**

In vitro cytotoxicity was tested against several cancer cell lines such as human lung (A549), human prostate (PC-3), human stomach (SNU-1), human liver (SNU-354), human breast (MCF7, MCF/ADR), NIH swiss mouse embryo fibroblast (NIH 3T3) and *ras* transformed NIH3T3 (F25), mouse fibroblast (L929), human oral epidermoid (KB-3-1) and its vinblastine selected multidrug resistance (KB-V1). All cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum. Cytotoxicity was measured using the SRB method (24) and their ED_{50} values were calculated by the Probit method. Antimicrobial activity was tested with agar dilution method (2). Bacteria were grown on Muller-Hinton agar medium, yeast were grown on YPD agar plate and fungi were grown on potato broth agar medium. Antimicrobial activity was observed after overnight incubation at 37°C for bacteria, 48 h incubation at 28°C for yeast and 5 days incubation at 25°C for fungi.

Polyclonal B Cell Activation by LPS

Spleen was removed aseptically from a female *Balb/c* mouse. Single cell suspensions were prepared in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). The density of lymphocytes was adjusted to 0.5×10^7 cells/ml. An aliquot (0.5 ml) of cell suspension was loaded to each well of a 48 well microplate and LPS was added at a final concentration of 25 μg/ml. Cells were incubated for 2 days at 37°C in humidified atmosphere of 7% O_2, 10% CO_2, and 83% N_2 with rocking of 7~10 complete cycles per minute (18). The number of antibody forming cells was determined by suspension hemolytic assay (10, 11). A target cell, trinitrophenol conjugated SRBC (TNP-SRBC), was prepared according to the previous method (20).

Mitogenic Response

Proliferation of spleen cells was determined by direct proliferation assay (26). The cells were suspended in the RPMI 1640 medium and dispensed to a cell density of 1×10^6 cells/ml in a 96 well plate. A test sample and 5 μg/ml of various appropriate mitogens such as Concanavalin A (ConA), Pokeweed mitogen (PWM), and LPS were added to each well at a concentration of 5 μg/ml. The cells were cultured for 72 h at 37°C in a humidified atmosphere of 5% CO_2. The [^{3}H] thymidine (1 μCi/well) was added to each well for the last 18 h of the incubation period and the amount of [^{3}H] thymidine incorporated into the cells was measured in a liquid scintillation counter.

RESULTS AND DISCUSSION

Physico-chemical Properties

The physico-chemical properties of dimethyl-elaiophylin and methyl-elaiophylin are summarized in Table 1. They were obtained as white powders and were soluble in MeOH and DMSO, insoluble in H_2O and C_6H_6. They showed similar R_f values of 0.22 and 0.25 on silica gel TLC plate when developed with $CHCl_3$-MeOH, 9 : 1.

Structure Determination

Dimethylelaiophylin was obtained as a white powder, mp. 188~189°C, and was optically active, $[\alpha]_D^{25}$ +9.96 (*c*

Table 1. Physico-chemical properties of dimethylelaiophylin and methylelaiophylin.

	Dimethylelaiophylin	Methylelaiophylin
Appearance	white powder	white powder
MP	188~189°C	153~155°C
$[\alpha]_D^{25}$ (MeOH)	+9.96 (*c* 1.06)	-15.46 (*c* 1.79)
UV λ_{Max}^{MeOH} nm (log ε)	253 (4.86)	252 (4.73)
Mass spectrum ESI-MS (*m/z*)	1075 $(M+Na)^+$	1061 $(M+Na)^+$
IR ν max(KBr) cm^{-1}	3472, 2972, 2939, 1705, 1639	3444, 2971, 2937, 1705, 1639
Molecular formula	$C_{56}H_{92}O_{18}$	$C_{55}H_{90}O_{18}$
Solubility		
Soluble	DMSO, $CHCl_3$, MeOH, EtOH	DMSO, $CHCl_3$, MeOH, EtOH
Slightly soluble	BuOH	BuOH
Insoluble	H_2O, Me_2CO, EtOAC, Hexane, Benzene	H_2O, Me_2CO, EtOAC, Hexane, Benzene
TLC (R_f)		
$CHCl_3$-MeOH (9 : 1)	0.22	0.25

1.06, MeOH). The observation of strong absorption at 3473 cm^{-1} and 1705 cm^{-1} in the IR spectra indicated the presence of the hydroxyl groups and the ester carbonyl groups in the molecules, respectively. A carbonyl group attached by a conjugated diene system was indicated by the IR (1639 cm^{-1}) and UV (253 nm) spectra. The ^{1}H-NMR spectrum revealed one characteristic methoxy peak at δ 3.04. The ^{13}C-NMR and DEPT spectra further revealed that this compound has 28 carbon signals containing 7 methyl, 3 methylene and 16 methine carbons. But the molecular ion peak in the ESI-MS spectrum appeared at *m/z* 1,075 $(M+Na)^+$, which was two fold more mass unit than the expected molecular ion. From the ^{13}C-NMR and MS data, we assumed that this compound might be a symmetrical macrolide antibiotic. Precise examinations of HOMOCOSY and HETEROCOSY spectra showed that these spectral data were in a good agreement with the 11, 11'-di-*O*-methylelaiophylin which had already been reported as a synthetic derivative of elaiophylin (21).

Monomethylelaiophylin was also obtained as white powder, mp 153~155°C, $[\alpha]_D^{25}$ -15.46 (*c* 1.79, MeOH). The UV, IR and ^{1}H-NMR spectra were similar with those of dimethylelaiophylin, so it was deduced to be an elaiophylin derivative. But ^{13}C-NMR spectrum showed 55 carbon signals suggesting that it was not a symmetrical structure (Table 2). The integration of the methoxyl signal in the ^{1}H-NMR spectrum corresponded to 3H, indicating that this compound had only one methoxyl group in the molecule. ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY spectra revealed the anomeric carbon peak at δ 93.3 and characteristic hemiketal carbon signals at δ 99.1 and δ 103.2. Such characteristic hemiketal formation in the structure was shown in oligomycins, azalomycins (8, 9), copiamycins (5) and methylconcanamycins (12). Unsymmetrical structure due to the one methoxyl group substituted at one hydroxyl group of C-11 gave a quite different chemical shift in values between the two hemiketal carbons (Δδ 4.0). The HMBC correlations of C-11 with H-9, H-10, H-12 and H-19 also confirmed the presence one methoxyl group attached at C-11. The molecular ion peak in the ESI-MS spectrum appeared at *m/z* 1,061 $(M+Na)^+$, which was exactly same as the value of the calculated molecular mass for methylelaiophylin. From the physico-chemical and all the spectroscopic data, the structure of this compound was determined as 11-*O*-

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

Position	δ C[a]		δ H[b]
1	169.4	170.0	–
2	121.3	121.0	5.68 (2H, m)
3	145.1	145.1	6.98 (2H, ddd, *J*=3.8, 11.4, 15.2 Hz)
4	131.7	132.0	6.12 (2H, m)
5	144.6	144.3	5.62 (2H, m)
6	40.9	41.3	2.53 (2H, m)
7	77.9	77.9	4.76, 4.93 (each 1H, brd)
8	35.9	36.7	1.89, 1.95 (each 1H, m)
9	69.6	70.0	3.46, 3.86 (each 1H, m)
10	38.0	41.6	1.72, 2.03 (each 1H, m)
11	103.2	99.1	–
12	34.3	34.3	1.46, 2.35 (each 2H, m)
13	70.2	70.7	3.95, 4.09 (each 1H, m)
14	47.4	48.4	1.20 (2H, m)
15	67.9	66.6	3.50, 3.94 (each 1H, m)
16	19.0	19.2	1.11, 1.19 (each 3H, d, *J*=6.3 Hz)
17	15.1	14.9	1.03, 1.05 (each 3H, d, *J*=6.6 Hz)
18	9.5	9.09	0.86, 0.88 (each 3H, d, *J*=6.8 Hz)
19	7.2	7.1	0.96, 1.00 (each 3H, d, *J*=7.3 Hz)
20	19.4	19.4	1.42, 1.62 (each 2H, m)
21	9.1	8.8	0.82, 0.86 (each 3H, d, *J*=6.9 Hz)
1'	93.3	93.3	5.05 (2H, d, *J*=7.2 Hz)
2'	33.4	33.5	1.80 (4H, m)
3'	65.9	66.0	3.96 (2H, m)
4'	71.4	71.5	3.61 (2H, brs)
5'	66.1	66.1	3.98 (2H, m)
6'	16.9	16.8	1.23, 1.24 (each 3H, d, *J*=6.8 Hz)
OCH_3	46.6		3.04 (3H, s)

[a]($CDCl_3$, 75 MHz), [b]($CDCl_3$, 300 MHz).

methylelaiophylin, which has not been reported yet. *O*-methylation of the hemiketal hydroxy group easily occurs in a methanol solution. Therefore, the acetone extract of mycelium was incubated with methanol overnight and the incubation mixture was analyzed by HPLC (solvent; 80% acetonitrile) in comparison with the acetone extract without incubation in methanol. This result indicated that dimethylelaiophylin and methylelaiophylin were indeed formed through *O*-methylation of the hemiketal hydroxyl group of elaiophylin during purification with a solvent containing methanol as reported by Hammann (6).

Cytotoxic and Microbial Activities

In vitro cytotoxicities of dimethylelaiophylin and methylelaiophylin were tested against several human and mouse cell lines using SRB assay (24). As shown in Table 3, methylelaiophylin and elaiophylin showed comparable cytotoxic activities against several cancer cell lines with adriamycin but dimethylelaiophylin was less active than methylelaiophylin and elaiophylin. The antimicrobial activities against bacteria, yeast and fungi were also determined by the agar dilution method (2). Dimethylelaiophylin showed no antimicrobial activity, and methylelaiophylin was only active against gram-positive bacteria (Table 4). In particular, methylelaiophylin showed much lower minimal inhibitory concentrations (MIC) than elaiophylin (15), suggesting a lack of symmetry in elaiophylin might be important for conferring antimicrobial activity.

Immune Suppression Activity

Many macrolide antibiotics such as FK506 (13), rapamycin (18), oligomycin F (14), and homooligomycin E (16) are well known to possess strong immunosuppressive activity. Concanamycin which contains the structural elements of elaiophylin was also reported as an effective inhibitor of the proliferation of mouse splenic lymphocytes and exhibited immunosuppressive activity (12). Therefore, three elaiophylins were evaluated for their immunosuppressive activities with lymphocytes prepared from mouse spleen. Various functions of lymphocytes, such as polyclonal B cell activation with lipopolysaccharide (LPS) and induced proliferation of cells by mitogens were determined. B cells were activated by the addition of LPS (25 μg/ml) for 2 days. The proliferation of lymphocytes was induced with 5 μg/ml of LPS, pokeweed mitogens (PWM) and concanavalin A (Con A) for 3 days. The antibody secretion was determined by a suspension hemolytic assay.

The results revealed that elaiophylin and methylelaiophylin dramatically suppressed B cell activation (97.5% and 97.6% suppression, respectively) and the induced proliferations of T and B cells by mitogens were also fully suppressed at a final concentration of 1 μg/ml (Fig. 2). However, dimethylelaiophylin exhibited no suppression on these parameters. These results indicate that elaiophylin and methyl-elaiophylin are potent immunosuppressive compounds which are comparable to cyclosporin A. Studies on the biological activities of elaiophylin and various synthetic derivatives of elaiophylin have indicated that symmetrical acetalization of elaiophylin led to a dramatic reduction of antibacterial activities and asymmetrical derivatives expressed anti-

Table 3. *In vitro* cytotoxic activity (ED_{50}, μM) against several cancer cell lines of dimethylelaiophylin and methylelaiophylin.

Cell line[a]	Dimethyl-elaiophylin	Methyl-elaiophylin	adriamycin
A 549	8.73	0.57	0.50
PC-3	1.79	1.54	1.31
NIH-3T3	2.66	1.45	0.17
F25	2.50	1.67	0.17
SNU-1	0.95	0.67	0.20
SNU-354	1.04	1.06	0.40
MCF-7	3.11	1.23	0.28
MCF-7/ADR	< 0.3	0.69	1.20
L929	5.83	3.26	1.15
KB-3-1	1.29	0.99	0.13
KB-V1	4.34	2.48	85.30

[a] A549, human lung carcinoma; PC-3, human prostate adenocarcinoma; MCF-7, human breast carcinoma; KB-3-1, human oral epidermoid carcinoma.

Table 4. Antimicrobial activities (MIC, μg/ml) of dimethylelaiophylin and methylelaiophylin.

Strains (ATCC#)		Dimethyl-elaiophylin	Methyl-elaiophylin
Staphylococcus aureus	6538P	> 50	1.56
Staphylococcus aureus	25923	> 50	1.56
Staphylococcus epidermidis	12228	> 50	1.56
Micrococcus luteus	9341	> 50	1.56
Enterococcus casseliflavus	14432	> 50	1.56
Enterococcus faecalis	29212	> 50	> 50
Bacillus subtilis	6633	> 50	1.56

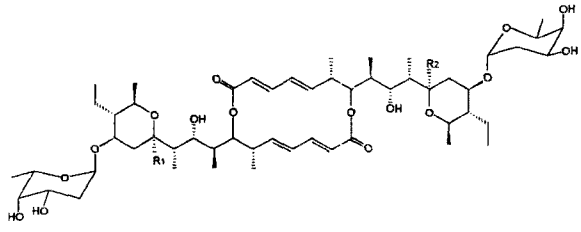

Fig. 1. Structures of dimethylelaiophylin, methylelaiophylin and elaiophylin.

compounds	R_1	R_2
dimethylelaiophylin	OCH_3	OCH_3
methylelaiophylin	OCH_3	OH
elaiophylin	OH	OH

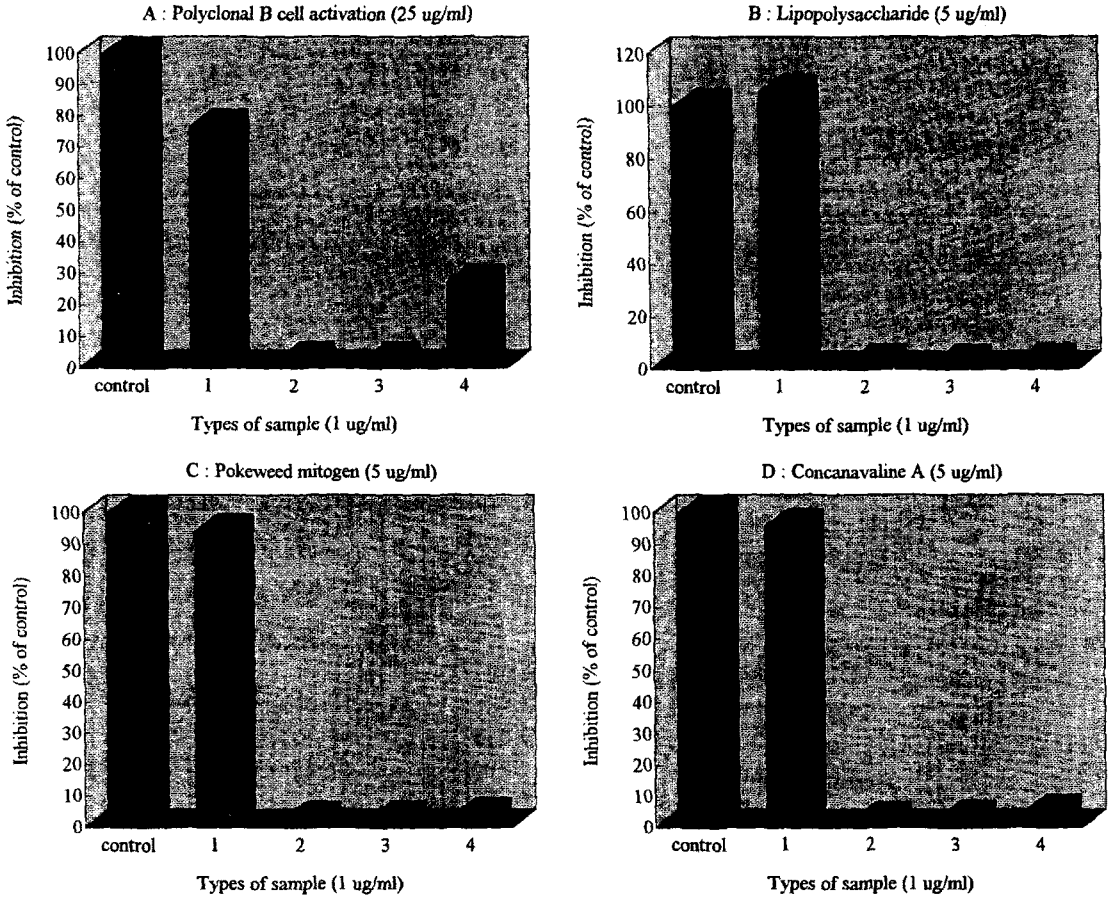

Fig. 2. Immunosuppressive activities of elaiophylins.
Lymphocytes were prepared from normal mouse spleens. Compounds were added to culture medium at a final concentration of 1 μg/ml. The polyclonal B cell activation (A) and the blastogenesis by LPS (B), PWM (C) and ConA (D) were determined. Results were calculated as percent inhibition to vehicle control (DMSO). The data represent mean ± standard deviation of six determinations. 1, dimethylelaiophylin; 2, methylelaiophylin; 3, elaiophylin; 4, cyclosporin A.

bacterial activity and stronger activity against nematodes (6). Our results showing the biological activities of elaiophylins also highlighted the lack of symmetry and/or a free OH group at C-11 of elaiophylin and that might be important for the biological activities.

Acknowledgement

This work was partially support by a grant from the Korean Ministry of Science and Technology.

REFERENCES

1. Arcamone, F. M., C. Bertazzoli, M. Ghione, and T. Scotti. 1959. Melanosporin and elaiophylin, new antibiotics from *Streptomyces melanosporus* (sive elanosporofaciens) n. sp. *Gion. Microbiol.* **7**: 207-216.
2. Barry, A. L., L. Creitz, and R. R. Packer. 1985. Feasibility study of disk diffusion susceptibility tests with Mueller-Hinton Broth solidified with gelrite, an agar substitute. *J. Clin. Microbiol.* **21**: 409-414.
3. Davies, M. G. and R. H. Green. 1986. Avermectins and milbemycins. *Nat. Prod. Rep.* **3**: 87-121.
4. Fiedler, H. P., W. Worner, H. Zahmer, H. P. Kaiser, W. Keller-Schierlein, and A. Muller. 1981. Metabolic products of M/Os. 200. Isolation and characterization of niphithricins A, B, and elaiophylin, antibiotics produced by *Streptomyces violaceoniger*. *J. Antibiot.* **34**: 1107-1118.
5. Fukushima, K., T. Arai, S. Iwasaki, M. Namikoshi, and S. Okuda. 1982. Studies on macrocyclic lactone antibiotics. VI. Skeletal structure of copiamycin. *J. Antibiot.* **35**: 1480-1494.
6. Hammann, P., G. Kretzschmar, and G. Seibert. 1990. Secondary metabolites by chemical screening. 7. I. Elaiophylin derivatives and their biological activities. *J. Antibiot.* **43**:

1431-1440.

7. Isogai, A., S. Sakuda, S. Matsumoto, M. Ogura, K. Furihata, H. Seto, and A. Suzuki. 1984. The structure of leucanicidin, a novel insecticidal macrolide produced by *Streptomyces halstedii*. *Agric. Biol. Chem.* **48**: 1379-1381.
8. Iwasaki, S., M. Namikoshi, K. Sasaki, K. Fukushima, and S. Okuda. 1982. Studies on macrocyclic lacton antibiotics. V. The structures of azalomycin F_{3a} and F5a. *Chem. Pharm. Bull.* **30**: 4006-4014.
9. Iwasaki, S., M. Namikoshi, K. Sasaki, M. Yano, K. Fukushima, S. Nozoe, and S. Okuda. 1982. Studies on macrocyclic lacton antibiotics. III. Skeletal structures of azalomycin F_{4a}. *Chem. Pharm. Bull.* **30**: 1669-1673.
10. Jerne, N. K. and A. A. Nordin. 1963. Plaque formation in agar by single antibody producing cells. *Science* **140**: 405.
11. Kim, D. H., K. H. Yang, K. W. Johnson, and M. P. Holsapple. 1987. Suppression of *in vitro* antibody production by dimethylnitrosoamine in mixed cultures of mouse primary hepatocytes and mouse splenocytes. *Toxicol. Appl. Pharmacol.* **87**: 32-42.
12. Kinashi, H., K. Someno, and K. Sakaguchi. 1984. Isolation and characterization of concanamycins A, B and C. *J. Antibiot.* **37**: 1333-1343.
13. Kino, T., H. Hatanaka, M. Hashimoto, M. Nishiyama, T. Goto, M. Okuhara, M. Kohsaka, H. Aoki, and H. Imanaka. 1987. FK-506, a novel immunosuppressant isolated from a *Streptomyces*. *J. Antibiot.* **40**: 1249-1255.
14. Laatsch, H., M. Kellner, G. Wolf, Y. S. Lee, F. Hansske, S. Konetschny-Rapp, U. Pessara, W. Scheuer, and H. Stockinger. 1993. Oligomycin F, a new immunosuppressive homologue of oligomycin A. *J. Antibiot.* **46**: 1334-1341.
15. Lee, S. Y., M. S. Kim, H. S. Kim, Y. H. Kim, S. D. Hong, and J. J. Lee. 1996. Structure determination and biological activities of elaiophylin produced by *Streptomyces* sp. MCY-846. *J. Microbiol. Biotechnol.* **6**: 245-249.
16. Lee, S. Y., S. B. Han, H. S. Kim, Y. H. Kim, H. M. Kim, C. J. Kim, S. D. Hong, and J. J. Lee. 1997. Immunosuppressive characteristics of oligomycin derivatives produced by *Streptomyces lydicus* MCY-524. *J. Microbiol. Biotechnol.* **7**: 56-61.
17. Lee, S. Y., S. C. Ha, Y. S. Hong, S. D. Hong, and J. J. Lee. 1997. Production of elaiophylin by the strain MCY-846 in a submerged culture. *J. Microbiol. Biotechnol.* **7**: 278-281.
18. Martel, R. R., J. Klicius, and S. Galet. 1977. Inhibition of the immune response by rapamycin, a new antifungal antibiotic. *Can. J. Physiol. Pharmacol.* **55**: 48-51.
19. Mishell, R. I. and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. *J. Med. Exp.* **217**: 423-433.
20. Rittenberg, M. B. and K. L. Pratt. 1969. Antitrinitrophenyl (TNP) plaque assay. Primary response of *Balb/c* mice to soluble and particulate immunogen. *Pro. Soc. Exp. Biol. Med.* **132**: 575-581.
21. Seebach, D., H. F. Chow, R. F. W. jackson, K. Lawson, M. A. Sutter, S. Thaisrivongs, and J. Zimmermann. 1985. Total synthesis of (+)-11, 11'-di-*O*-methylelaiophylidene, an aglycon of elaiophylin. *J. Am. Chem. Soc.* **107**: 5292-5293.
22. Seto, H., H. Akao, K. Furihata, and N. Otake. 1982. The structure of a new antibiotic, hygrolidin. *Tetrahedron Lett.* **23**: 2667-2670.
23. Skehan, P., R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, and M. R. Boyd. 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer. Inst.* **82**: 1107-1112.
24. Toshima, K., K. Tatsuta, and M. Kinoshita. 1986. Total synthesis of elaiophylin (azalomycin B). *Tetrahedron Lett.* **27**: 4741-4744.
25. Werner, G., H. Hagenmaier, H. Drautz, A. Baumgartner, and H. Zahner. 1984. Metabolic products of microorganisms. 224. Bafilomycins, a new group of macrolide antibiotics. Production, isolation, chemical structure and biological activity. *J. Antibiot.* **37**: 110-117.
26. Wood, S. C., J. G. Karras, and M. P. Holsapple. 1992. Integration of the human lymphocyte into immunotoxicological investigations. *Fundament. Appl. Toxicol.* **18**: 450-459.

(Received April 28, 1997)