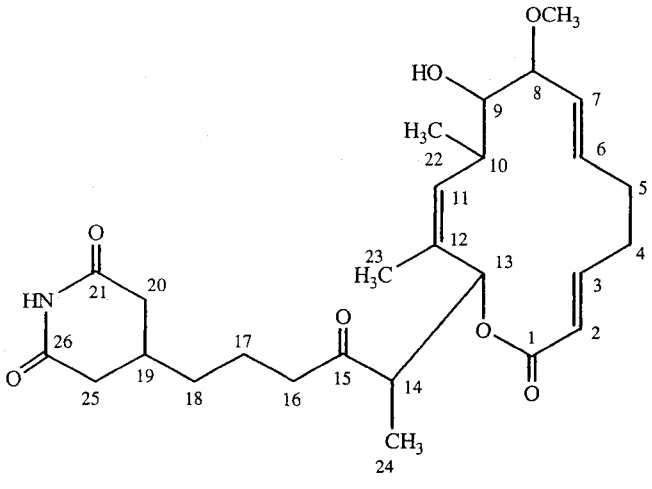
Migrastatin, a Novel 14-Membered Lactone from *Streptomyces* sp. MK929-43F1

Sir:

We have isolated migrastatin (Fig. 1), as an inhibitor of tumor cell migration, from a cultured broth of *Streptomyces* sp. MK929-43F1. In the preceding paper[1], the taxonomy, fermentation, isolation and biological activities were reported. In this paper, we describe the physico-chemical properties and structure elucidation of migrastatin.

Physico-chemical properties of migrastatin are summarized in Table 1. Migrastatin is readily soluble in methanol, acetone, ethyl acetate, chloroform and DMSO and practically insoluble in water and *n*-hexane. Migrastatin was isolated as a white powder with melting point of 54~55°C. The UV spectrum showed end absorption. Migrastatin gave positive color reaction with molybdophosphoric acid-sulfuric acid and 2,4-dinitrophenylhydrazine but negative with ninhydrin and Rydon-Smith. The molecular formula for migrastatin was established as $C_{27}H_{39}NO_7$ by HRFAB-MS, which was supported by the ^1H and ^{13}C NMR spectra. The DEPT and HMQC spectra exhibited 27 resonances; three methyl, one methoxy, seven methylene, five methine, one oxygen bearing methine, five olefinic methine, one quaternary olefinic and four carbonyl carbons. The ^1H NMR spectrum exhibited the presence of two deuterium exchangeable protons other than the protons which were attributed to the carbons described above. These ^1H and ^{13}C chemical shifts are listed in Table 2.

Fig. 1. Structure of migrastatin.



In the C1~C13 unit of the molecule, two substructures, CH=CH–CH_2 (C2~C4) and CH_2–CH=CH–CH–CH–CH–CH=C (C5~C11) were elucidated by the ^1H-^1H COSY spectrum. Additional informations from the HMBC spectrum; the long-range couplings from a methoxy group to C-8, from a hydroxy group to C-9 and from a methyl group at C-12 (H-23) to C-11, 12 and 13 suggested fully substituted partial structure, C-5~C-13. Another informations; the long-range couplings from the 13-H and the 2-H to a carbonyl carbon at C-1 (δ_C 163.4) whose chemical shift was assignable to α,β-unsaturated ester carbonyl, suggested the bonds between C-13 and C-2 through –OCO–. The bond between C-4 and C-5 was not obvious by these experiments because of the signal overlapping. This was determined by a HOHAHA spectrum, which showed a spin system from the 3-H to 6-H and 7-H. Thus, the 14-membered lactone ring for the C1~C13 unit was established.

The side chain part was determined in the same manner as described above. The ^1H-^1H COSY spectrum exhibited two substructures, C(13)H–CH–CH_3 (C13~C14) and CH_2–CH_2–CH_2–CH–$(CH_2)_2$ (C16~C20/25). In the HMBC spectrum, the long-range couplings from H-14 and H-16 to a carbonyl carbon at C-15 (δ_C 210.8) whose chemical shift

Table 1. Physico-chemical properties of migrastatin.

Appearance	white powder
Nature	neutral
mp	54~55°C
$[\alpha]_D^{27}$	+17.9° (*c* 3.18, MeOH)
Molecular formula	$C_{27}H_{39}NO_7$
FAB-MS (*m/z*)	490 $(M+H)^+$
	488 $(M-H)^-$
HRFAB-MS (*m/z*)	
Calcd	490.2805 $(M+H)^+$
Found	490.2775 $(M+H)^+$
UV in MeOH	end
IR λ max (KBr) cm^{-1}	3207,2933,1718,1698,1260
R_f value[a]	0.51

[a]Silica gel TLC (Merck Art. 1.05715, $CHCl_3$:MeOH=10:1)

Dedicated to the memory of Sir EDWARD ABRAHAM.

Table 2. ^{13}C and ^{1}H data for migrastatin in $CDCl_3$.

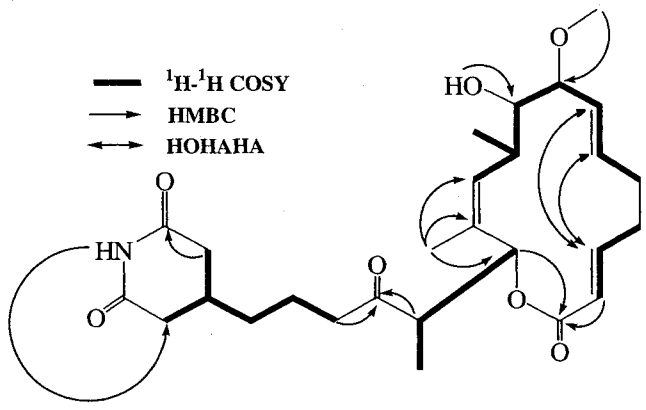
Position	δ_C (ppm)	δ_H (ppm)
1	163.4	
2	122.2	5.59 (1H, dd, 15.7, 1.5)
3	150.0	6.50 (1H, ddd, 15.7, 10.3, 3.6)
4	31.10	2.20~ 2.30 (1H, m)
		2.40~ 2.45 (1H, m)
5	31.14	2.20~ 2.30 (1H, m)
		2.40~ 2.45 (1H, m)
6	130.6	5.52 (1H, ddd, 15.5, 9.2, 4.6)
7	128.1	5.24 (1H, dd, 15.5, 4.7)
8	82.5	3.47 (1H,dd, 8.6, 4.7)
9	78.0	3.05 (1H, d, 8.6)
10	32.0	2.89~2.96 (1H, m)
11	133.1	5.65 (1H, dd, 10.6,1.5)
12	131.2	
13	77.0	5.10 (1H, d, 10.3)
14	51.2	2.94 (1H, dq, 10.3, 7.0)
15	210.8	
16	40.0	2.50 (2H, t, 7.0)
17	20.2	1.59~1.65 (2H, m)
18	34.2	1.32~1.38 (2H, m)
19	30.4	2.08~2.17 (1H, m)
20	37.7	2.20~ 2.30 (1H, m)
		2.65~ 2.75 (1H, m)
21	171.8	
22	13.4	0.96 (3H, d, 7.0)
23	26.0	1.88 (3H, d, 1.5)
24	13.4	1.12 (3H, d, 7.0)
25	37.7	2.20~ 2.30 (1H, m)
		2.65~ 2.75 (1H, m)
26	171.8	
8-OCH_3	57.0	3.31 (3H, s)
NH		7.93 (1H, brs)
OH		2.82 (1H, brs)

Chemical shifts in ppm form TMS as internal standard

was characteristic to a ketone carbonyl, showed the connectivity between the two substructures mentioned above through –CO–. The two methylenes (C-20 and C-25) and two carbonyls (C-21 and C-26, δ_C 171.8) in the terminal of the side chain are spectroscopically equivalent. Additionally, the long-range couplings from the H-20/25 to carbonyl carbons at C-21/26 and the remaining NH to C-20/25 suggested the existence of a symmetrical glutarimide structure. Key informations from ^{1}H-^{1}H COSY, HMBC, HOHAHA spectra are summarized in Fig. 2.

Geometries for the two olefins at C-2 and C-6 were revealed to be 2*E* and 6*E* by their large spin coupling,

Fig. 2. ^{1}H-^{1}H COSY, HMBC and HOHAHA correlation of migrastatin.



$J_{2,3}$=15.7 Hz and $J_{6,7}$=15.5 Hz, respectively. A significant NOE between the 11-H and 23-H was observed in the NOESY spectrum indicating that the trisubstituted olefin was of *Z* stereochemistry. From the all above described results, the structure of migrastatin was determined as shown in Fig. 1.

The absolute stereochemistry of migrastatin is now in progress.

Acknowledgment

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References

1) NAKAE, K.; Y. YOSHIMOTO, T. SAWA, Y. HOMMA, M. HAMADA, T. TAKEUCHI & M. IMOTO: Migrastatin, a new inhibitor of tumor cell migration from *Streptomyces* sp. MK929-43F1. Taxonomy, fermentation, isolation and biological activities. J. Antibiotics 53: 1130~1136, 2000