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Migrastatin and a New Compound, Isomigrastatin, from *Streptomyces platensis*

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Streptomyces platensis (strain NRRL 18993), a producer of dorrigocins, was shown to produce migrastatin, a cyclic congener of dorrigocin A previously reported from a different organism. Additionally a new compound isomeric to migrastatin, isomigrastatin, was also isolated and its structure was determined to be a cyclic form of dorrigocin B. Both compounds were fully characterized from MS and NMR data. Product titers of both were improved by the addition of XAD-16 resin to the fermentation medium.

Development of a treatment to prevent the metastasis of tumor cells is essential to stopping tumor dissemination[1]. One potential therapeutic agent is migrastatin (Fig. 1), a non-cytotoxic glutarimide-containing compound possessing a 14-membered lactone ring, which has been reported to inhibit *in vitro* migration of human esophageal cancer EC17 cells and mouse melanoma B16 cells[2,3]. Migrastatin was first isolated from cultures of *Streptomyces* sp. MK929-43F1[2].

Streptomyces platensis produces the dorrigocins (Fig. 1), which are isomeric acyclic compounds derivable from migrastatin[4,5]. While *S. platensis* has not previously been reported to produce migrastatin, because of its structural similarities to dorrigocin A, we believed that *S. platensis* may also produce migrastatin (Fig. 1). Here we show that *S. platensis* does indeed produce migrastatin, as well as a new related compound isomigrastatin, a cyclic form of dorrigocin B. The production, purification, and characterization of migrastatin and isomigrastatin from fermentations of *S. platensis* are described.

Materials and Methods

General

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ solution at 300 K with a Bruker DRX 400 spectrometer equipped with a QNP z-axis gradient probehead. Chemical shifts were referenced to δ 7.26 and 77.0 for ^{1}H and ^{13}C spectra, respectively. Cultures were screened for metabolite production on a PESciEx API100LC single quadrupole LC/MS spectrometer configured with an atmospheric pressure chemical ionization (APCI) source in positive-ion mode with a ring voltage of 250, orifice voltage of 20, and a source temperature of 350°. HR-LC-MS spectra were measured on an Applied Biosystems Mariner time-of-flight mass spectrometer equipped with a turbo-ionspray source. The spray chamber temperature was 400°C and the nozzle potential was 120 V. The spray tip potential was 5400 V for positive ion, and −4500 V for negative ion measurements. Dansyl-Gly-Trp was used as a mass standard internal to the samples.

Strains and Culture Conditions

Streptomyces platensis NRRL 18993 was used for this work. Cell banks were prepared by adding glycerol (30% v/v final concentration) to a culture growing exponentially in inoculum medium and then freezing 1 ml samples at −80°C. Inoculum medium[4] consisted of 15 g/liter soy flour (Giusto's Vita-Grain), 1 g/liter yeast extract (Sigma), 1 g/liter NaCl, and 1 g/liter $CaCO_3$ in deionized water. The pH was adjusted to 7.0 with 2.5 N NaOH and the medium was autoclaved for 60 minutes at 121°C. After cooling,

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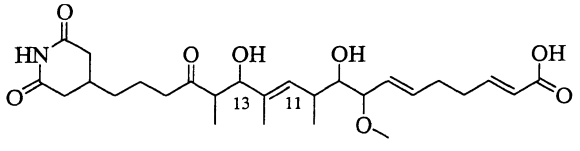
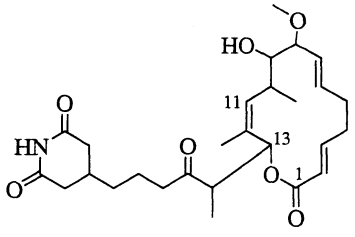
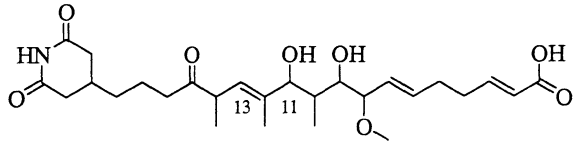
Fig. 1. Structures of dorrigocin A, migrastatin, dorrigocin B, and isomigrastatin.



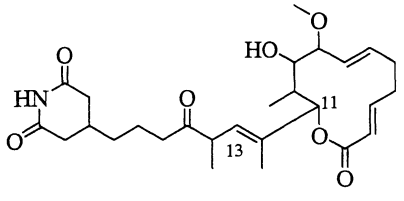
dorrigocin A



migrastatin



dorrigocin B



isomigrastatin

sterile glucose solution (500 g/liter) was added to give a final concentration of 13.6 g/liter. Production medium[4] contained 100 g/liter XAD-16 (Amberlite), 20 g/liter soy flour, 1 g/liter yeast extract, and 2 g/liter $CaCO_3$. The pH was adjusted to 7.0 with 2.5 N NaOH and the medium was autoclaved for 60 minutes at 121°C. After cooling, sterile glucose solution (500 g/liter) was added to give a final concentration of 18.2 g/liter. One cell bank vial was used to inoculate 50 ml of inoculum medium in a 250 ml baffled flask. The culture was allowed to grow for 2~3 days at 250 RPM and 28°C. It was then used to inoculate flasks containing 50 ml production medium at 5% of the final volume. Production flasks were incubated at 28°C on a 250 RPM rotary shaker for 8 days. For fermentor studies, the 50 ml culture was transferred to 500 ml of inoculum medium in a 2.8-liter Fernbach flask, allowed to grow for 2 days, and used to inoculate 10-liter fermentors containing production medium at 5% v/v. Inoculum cultures for a 150-liter fermentation were started as described above. To further expand the inoculum, the 500 ml inoculum culture was subcultured into a 10-liter fermentor containing 5 liters inoculum medium grown for 2 days, and then used to inoculate a 150-liter fermentor containing 100 liter production medium at 5% v/v.

Fermentations were controlled at 28°C and pH 7.0 by addition of 2.5 N H_2SO_4 and 2.5 N NaOH. Foaming was controlled by the automatic addition of 50% (v/v) Antifoam B (J.T. Baker). Dissolved oxygen was maintained above 50%, with an agitation rate between 500~550 RPM and aeration rate between 0.1~0.3 VVM. Cultures were harvested after 8~9 days.

Extraction and Analysis

Cultures (50 ml) were centrifuged at 3313×*g* for 10 minutes. The supernatant was decanted, and the cell pellet together with the XAD was washed with water. After the water wash, 25 ml of MeOH was added to the pellet and the mixture was placed on a 175 RPM shaker for 30 minutes at 30°C. The MeOH was decanted and reserved. The extraction was repeated and the MeOH extracts were pooled, evaporated to solids, and resuspended in 2 ml of MeOH for LC-MS or HPLC analysis. In general, samples were chromatographed using a 4.6×150 mm Inertsil 5 μm ODS-3 column with 48% MeCN - H_2O (0.1% acetic acid) over 13 minutes at a flow rate of 1 ml/minute with UV detection at 205 nm. Mass spectra for both compounds with an APCI source, positive ion mode: *m/z* 528.3, 512.3, 507.3, 490.3, 472.3, 458.3, 440.3, 422.2, 292.2, 247.1.

For HR-TOF-LC-MS, a linear gradient of 15% MeCN - H_2O (0.1% AcOH) to 70% MeCN - H_2O (0.1% AcOH) over 12 minutes, 0.4 ml/minute, on an Inertsil 5 μODS-3 column (2.1×150 mm) was used. HR-LC-TOF-MS for *m/z* 488 at 12.2 minutes: $[M-H]^-$ calculated for $C_{27}H_{38}NO_7$ (isomigrastatin), 488.2643; found, 488.2668. HR-LC-TOF-MS for *m/z* 488 at 10.3 minutes: $[M-H]^-$ calculated for $C_{27}H_{38}NO_7$ (migrastatin), 488.2643; found, 488.2628. HR-LC-TOF-MS for *m/z* 506 at 8.4 minutes: $[M-H]^-$

calculated for $C_{27}H_{40}NO_8$ (dorrigocins), 506.2748; found 506.2688. HR-LC-TOF-MS for *m/z* 506 at 8.0 minutes: $[M-H]^-$ calculated for $C_{27}H_{40}NO_8$ (dorrigocins), 506.2748; found 506.2695.

Purification

Material purified from two 10-liter fermentations was used to confirm the production of migrastatin by *S. platensis*. The XAD-16 resin (1.5 liters) in 16 liters of fermentation broth was captured using an 8.9×33 cm column. Migrastatin was eluted with 10 liters of MeOH at a flow rate of 320 ml/minute. The product-containing solution was evaporated to solids. The solids were extracted with 250 ml of MeOH and then filtered. The filtered solids were washed with an additional 50 ml of MeOH. The wash and filtrate were combined and diluted with 700 ml of water. This solution was loaded onto a 4.8×25 cm C18 column and eluted using a step gradient from 30~100% MeOH in water at a flow rate of 100 ml/minute. Eight 1-liter fractions were collected, and the migrastatin was found exclusively in fraction 4, which had been collected during elution with 60% MeOH. Fraction 4 was evaporated to an oil. The oil was redissolved in 100 ml of MeOH, and the total volume was brought up to 250 ml with water. The solution was loaded onto a 2.5×20 cm C18 column at a flow rate of 25 ml/minute. The column was eluted with 40% MeOH (800 ml, Fraction 1), 42% MeOH (800 ml, Fraction 2), and 44% MeOH (4 liters, Fractions 3~32). Fractions 13~20 were combined and the product pool was evaporated to yield migrastatin as an oil.

Isomigrastatin was purified from approximately half of a 150-liter fermentation for structural determination. XAD-16 (10 liter) was captured from 100 liters of fermentation culture in a 10-liter column. Isomigrastatin was eluted with 90 liters of MeOH and the eluate was evaporated to solids. A portion (15.4 g) of the solids was dissolved with 250 ml of MeOH, and the mixture was stirred at room temperature for two hours. This suspension was filtered and diluted to 50% MeOH. The mixture was loaded onto a 2.5×15 cm C18 column that had been equilibrated with 50% MeOH. The column was washed with 50% MeOH and eluted with 60% MeOH. Fractions containing isomigrastatin were evaporated to dryness, and the residue was extracted with 3×100 ml acetone. The acetone solution was dried to solids and further dried in a vacuum oven at 40°C and 30 mbar. The resulting solids were purified by preparative HPLC using a Metachem Polaris 10 μm C18-A (21.2×250 mm) column with 50% MeCN as the eluting solvent at 20 ml/minute to give 32 mg of isomigrastatin as an oil.

Results and Discussion

Detection of Dorrigocins and Migrastatins

Cultures of *S. platensis* were examined for the presence of migrastatin and dorrigocins under several LC-MS conditions. High resolution negative ion time-of-flight LC-MS measurements of extracts indicated the presence of compounds with the elemental composition of dorrigocins, as well as two compounds with the elemental composition of migrastatin. The mass spectra of both migrastatin isomers gave extensive and essentially identical fragmentation with an atmospheric pressure chemical ionization source in positive-ion mode. The earlier eluting isomer was purified from two 10-liter fermentations and confirmed to be migrastatin by comparing its ^1H and ^{13}C NMR data (Table 1) to that reported by NAKAE *et al.*[3].

Fermentation Studies

KARWOWSKI *et al.*[4] found that including XAD-16 in the production medium seemed to stabilize the structurally similar compounds dorrigocin A and B in culture broth. To assess whether XAD-16 resin had a similar stability affect on migrastatin, an XAD resin study was performed in shake flasks examining the effects of 5, 10, 20, 50, and 100 g/liter XAD-16 on production titers (Fig. 2). Although XAD-16 is reported here, other hydrophobic resins (*e.g.*, HP20, Mitsubishi) demonstrated similar effects (data not shown). Fig. 2 shows that the addition of 100 g/liter XAD-16, as compared to 5 g/liter XAD-16, to the production medium increased isomigrastatin titers tenfold and migrastatin titers by 15%. In subsequent large scale fermentations, use of 100 g/liter XAD-16 was consistently found to have a stimulatory effect on production, probably through a stability effect on the migrastatin and isomigrastatin[6]. It is also possible that the migrastatins are toxic to the cells above a certain level, and thus sequestering the compounds from the broth increases titers[7]. XAD-16 may also bind certain nutrients in the fermentation broth and thus influence secondary metabolism[8].

Migrastatin and isomigrastatin were produced in similar ratios in the 10-liter and 150-liter fermentations. Production of the migrastatins reached a maximum on day 7 in the 150-liter fermentation (Fig. 3). The culture was harvested from the 150-liter fermentation on day 9.

Structure Elucidation of Isomigrastatin

Isomigrastatin was purified from approximately half of

Table 1. ^{1}H and ^{13}C chemical shifts of migrastatin and isomigrastatin.

Position	migrastatin		isomigrastatin	
	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
1	163.9	--	167.6	--
2	122.1	5.56, 1H, dd (*J*=15.5, 1.5)	124.9	5.66, 1H, d (*J*=16.0)
3	150.0	6.47, 1H, ddd (*J*=15.5, 10.0, 3.5)	150.6	6.63, 1H, ddd (*J*=16.0, 8.5, 7.0)
4	31.0	2.20, 1H, m	30.0	2.15, 1H, m
		2.40, 1H, m		2.45, 1H, m
5	31.9	2.20, 1H, m	32.7	1.96, 1H, m
		2.40, 1H, m		2.60, 1H, m
6	130.5	5.50, 1H, ddd (*J*=15.5, 9.5, 5.0)	129.1	5.58, 1H, ddd (*J*=15.5, 11.0, 4.0)
7	128.0	5.22, 1H, dd (*J*=15.5, 5.0)	130.3	5.07, 1H, dd (*J*=15.5, 3.5)
8	82.5	3.46, 1H, dd (*J*=8.5, 5.0)	81.6	3.40, 1H, m
9	77.9	3.02, 1H, dd (*J*=8.5, 1.5)	73.1	3.72, 1H, d (*J*=9.0)
10	31.9	2.90, 1H, m	38.0	1.83, 1H, m
11	133.0	5.63, 1H, dd (*J*=10.5, 1.5)	82.8	5.20, 1H (overlap)
12	131.1	--	134.0	--
13	76.9	5.07, 1H, d (*J*=10.5)	127.9	5.20, 1H (overlap)
14	51.1	2.95, 1H, dd (*J*=10.5, 7.0)	45.9	3.40, 1H, m
15	210.9	--	210.8	--
16	40.0	2.48, 2H, t (*J*=7.0)	39.9	2.60, 1H, m
				2.38, 1H, m
17	20.1	1.59, 2H, m	20.2	1.57, 2H, m
18	34.1	1.32, 2H, m	34.1	1.33, 2H, m
19	30.3	2.10, 1H, m	30.0	2.10, 1H, m
20	37.6	2.20, 1H, m	37.8	2.25, 1H, m
		2.67, 1H, m		2.70, 1H, m
21	172.1	--	172.2	--
22	13.3	0.94, 3H, d (*J*=7.0)	10.5	0.85, 3H, d (*J*=7.0)
23	25.9	1.83, 3H, d (*J*=1.5)	13.3	1.88, 3H, s
24	13.3	1.10, 3H, d (*J*=7.0)	15.7	1.11, 3H, d (*J*=6.5)
25	37.6	2.20, 1H, m	37.8	2.25, 1H, m
		2.67, 1H, m		2.70, 1H, m
26	172.1	--	172.2	--
8-OCH_3	56.9	3.28, 3H, s	57.1	3.31, 3H, s

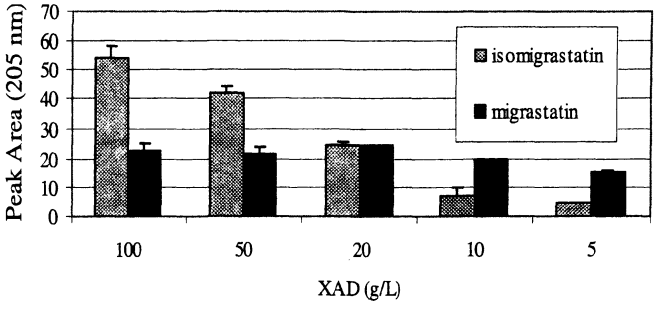
the 150 liter fermentation. Its structure was determined by MS and NMR methods (^{1}H, ^{13}C, COSY, pure absorption TOCSY, HMBC, and multiplicity-edited HSQC) and by comparison with data reported for the known compounds migrastatin, dorrigocin A, and dorrigocin B (Table 1, Fig. 5). COSY and HMBC correlations placed a double bond between C-12 and C-13 as observed in dorrigocin B. The chemical shift of C-23 indicated that the double bond at C-12 was of *E* configuration, as is the configuration at this position of dorrigocin B. An HMBC correlation from H-11 to C-1 confirmed the 12-membered lactone ring of isomigrastatin. The stereochemistry of the dorrigocins and migrastatins remains to be determined.

Conclusion

In addition to dorrigocins, *Streptomyces platensis* produces migrastatin and a new related polyketide, isomigrastatin. Production of the migrastatins leveled off after 7 days. XAD-16 was used in the fermentation to improve titers as well as to stabilize both molecules.

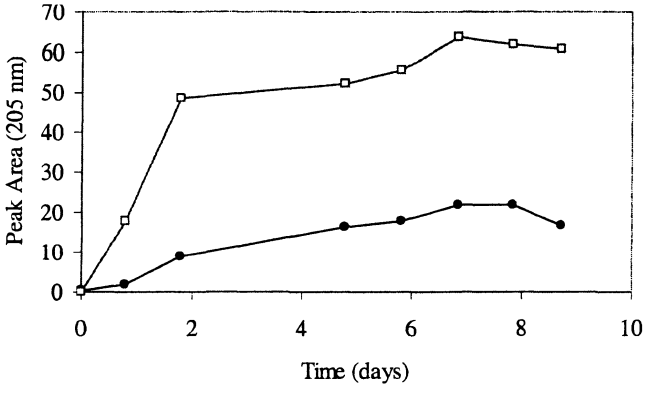
Dorrigocins A and B differ by the orientation of an allylic alcohol at C-11 through C-13. While dorrigocin B is

Fig. 2. Effect of XAD-16 concentration on production of migrastatin and isomigrastatin.



Bars represent titers after 8 days in shake flasks. Titers are reported as UV peak areas at 205 nm.

Fig. 3. Time course of migrastatin (●) and isomigrastatin (□) production in a 150-liter fermentor.



Titers are reported as UV peak areas at 205 nm.

Fig. 4. Chromatogram of XAD-16 eluate indicating migrastatin and isomigrastatin (UV 205 nm using 48% MeCN - H_2O with 0.1% acetic acid over 13 minutes).

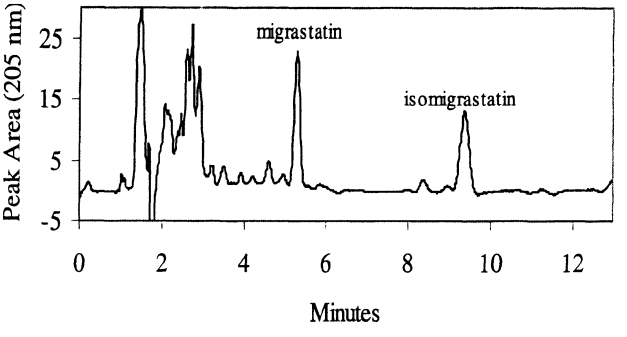
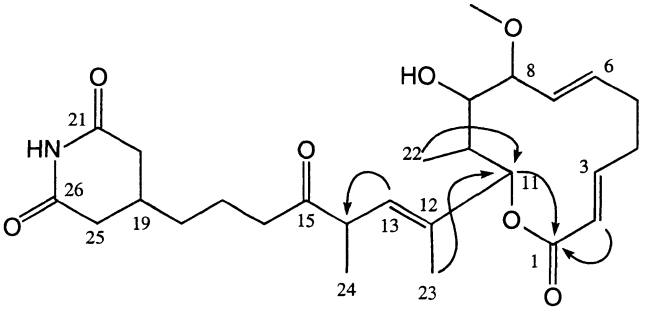


Fig. 5. Key HMBC correlations observed for isomigrastatin.



Arrows point from protons to carbons.

derivable directly by hydrolysis of isomigrastatin, direct conceptual hydrolysis of migrastatin leads to an acyclic analogue of dorrigocin A with a *cis* C-12, C-13 double bond. The precise origin and derivation of this group of metabolites remain to be elaborated.

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