A80915, A NEW ANTIBIOTIC COMPLEX PRODUCED BY *STREPTOMYCES ACULEOLATUS*

DISCOVERY, TAXONOMY, FERMENTATION, ISOLATION, CHARACTERIZATION, AND ANTIBACTERIAL EVALUATION†

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New semi-naphthaquinone antibiotics A80915A, B, C, and D were isolated from the fermented broth of *Streptomyces aculeolatus* A80915 (NRRL 18422). Factors A and C, present in both the broth filtrate and mycelial methanol extract, and factors B and D, found predominantly in the broth filtrate, were recovered by extraction with ethyl acetate. Purification of the individual factors was accomplished by preparative reverse phase high performance liquid chromatography on C_{18} bonded silica supports. Factors A~D show antimicrobial activity against Gram-positive aerobic and anaerobic organisms *in vitro*. Mechanism of action studies demonstrated nearly complete inhibition of macromolecular biosynthesis (protein, RNA, DNA, and cell wall) by A80915 factors A~D. A less highly cyclized semi-naphthaquinone, A80915 factor G, was isolated from the broth of the strain fermented in an alternate medium.

Strain A80915, identified as *Streptomyces aculeolatus*, was isolated from a soil sample collected in 1981 from the Palau Islands, Western Caroline Islands. The culture produces a complex of Gram-positive antibiotics related to the napyradiomycin[1~4] and SF2415[5,6] antibiotics. Under certain culture conditions strain A80915 can produce tetronomycin[7], a polyether antibiotic, or SF2415-type antibiotics with little or no napyradiomycin-type antibiotics. This communication describes the taxonomy of the producing strain, fermentation conditions employed for biosynthesis, and the isolation, characterization, and antibacterial evaluation of five semi-naphthaquinone factors (A, B, C, D, and G) produced by the strain. The structure elucidation of these antibiotics will be reported separately.

Materials and Methods

General Methods

UV spectra were run on a Cary model 118 spectrophotometer. IR spectra were recorded on a Nicolet

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MX-1 FT-IR spectrometer. Optical rotations were determined on a Perkin-Elmer model 241 polarimeter. FAB-MS were run on a Varian-MAT 731 or a VG Analytical ZAB 3 mass spectrometer; FD-MS were run on the Varian-MAT 731.

Fermentation

The A80915 strain was inoculated into the two fermentation media described in Table 2 and cultured at 30°C on a conventional rotary shaker (250 rpm, 6.4-cm throw). When desired, K_2HPO_4 was added to the fermentation medium prior to autoclaving. Phosphate (as inorganic phosphorus) was estimated on the IL Monarch Chemistry System. The phosphate concentration in M1 and M2 media was usually 35~40 and 5~10 μg/ml, respectively.

HPLC Assay of A80915 Factors

Individual A80915 factors were identified and quantitated by analytical HPLC on a Beckman Ultrasphere C_{18} column (4.6 mm × 25 cm, 5 μm). The mobile phase consisted of THF - MeOH - CH_3CN - H_2O - H_3PO_4 (30 : 50 : 5 : 14.9 : 0.1) at a flow rate of 1.5 ml/minute. UV absorption was monitored at 252 nm.

Isolation of A80915 Complex Produced in Medium M1

Whole broth (110 liters) produced by fermentation of strain A80915 in medium M1 was filtered through a filter press with 5% Hyflo Super Cel filter aid. The mycelial cake was extracted with acetone (30 liters), which was concentrated to aqueous solution (6 liters) and then extracted with EtOAc (2 × 3 liters). The ethyl acetate extracts were combined, washed with water, and concentrated to give 75.4 g of dried mycelial extract. The broth filtrate was extracted with EtOAc (40 liters). The ethyl acetate extract was backwashed with 400 ml of water and then concentrated to give 72.85 g of dried broth extract.

Purification of A80915 Factors A and C from Dried Mycelial Extract

Ethyl acetate extract of the mycelial methanol extract (40 g) was subjected to preparative reverse phase HPLC (RPHPLC) on a column (4.7 × 60 cm) packed with 20 μm octadecylsilanized Quantum LP-1 silica gel (LP-1/C_{18}). Elution with CH_3CN - H_2O - HCOOH (80 : 19.9 : 0.1) gave A80915C (1.691 g). Further elution with CH_3CN yielded impure A80915A (1.994 g), which was combined with 0.7 g of comparably impure A from a similar workup. Further preparative RPHPLC on columns (2~2.2 × 30 cm) of DuPont Zorbax ODS (12 μm), eluting with CH_3CN - H_2O - HCOOH (70 : 29.8 : 0.2), yielded A80915A (749 mg).

Purification of A80915 Factors A~D from Dried Broth Extract

Ethyl acetate extract of the broth filtrate was subjected to preparative RPHPLC on a column (4.7 × 60 cm) packed with LP-1/C_{18} (20 μm). Elution with CH_3CN - H_2O - HCOOH (70 : 29.9 : 0.1) yielded A80915D (1.322 g). Continued elution with CH_3CN - H_2O - HCOOH (75 : 24.9 : 0.1) yielded A80915C (0.918 g) followed by A80915B (1.105 g). Impure A80915A (0.631 g) was removed from the column with 100% CH_3CN and combined with 225 mg of comparably impure A from a similar workup. Further preparative RPHPLC on a column (2.2 × 30 cm) of DuPont Zorbax ODS (12 μm), eluting with CH_3CN - H_2O - HCOOH (90 : 9.9 : 0.1), yielded A80915A (282 mg).

Isolation and Purification of A80915 Factor G

Whole broth (10 liters) produced by fermentation of strain A80915 in medium M2 was suction filtered with Hyflo Super cel filter aid. The broth filtrate (pH 7.05) was extracted three times with EtOAc (6, 4, and 4 liters). The combined EtOAc extracts were filtered and concentrated to dryness to give 0.7 g of dried filtrate extract. The mycelial cake was extracted with MeOH (2 × 1.5 liters). The MeOH extract was concentrated and lyophilized and then partitioned between H_2O (100 ml) and EtOAc (500 ml). The EtOAc layer was concentrated to give 1.5 g of dried extract. The dried mycelial extract was subjected to preparative RPHPLC on a Rainin Dynamax-60A C18 column (41.4 × 30 cm, 8 μm) coupled to a column (22 mm × 30 cm) packed with Amicon Matrex silica LC (10 μm) packing. Elution with mobile phase gradients of CH_3CN - MeOH - H_2O - HCOOH from 40 : 20 : 39.9 : 0.1 to 60 : 30 : 9.9 : 0.1 to 95 : 0 : 4.9 : 0.1 at a flow rate of 20 ml/minute yielded SF2415B1 (111 mg), A80915G (18.2 mg), SF2415A2 (76 mg), and SF2415B2 (32 mg).

Methods for the Evaluation of Antibacterial Activity and Toxicity

All bacterial strains used in this study were clinical isolates obtained from numerous sources of broad geographic distribution. Isolates were maintained frozen in liquid nitrogen or in $-70°C$ electric freezers.

MICs were determined by an agar dilution method in accordance with the procedures outlined by the National Committee for Clinical Laboratory Standards†. Inocula were adjusted to yield approximately 10^{-5} cfu per spot. Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, MD.) was used for all of the aerobic organisms tested. Schadler agar (Difco Laboratories, Detroit, MI.) was used for all of the anaerobic organisms. The MIC was considered to be the lowest concentration that either prevented visible growth or gave rise to fewer than three discrete colonies.

Mouse protection tests were performed against one strain of *Staphylococcus aureus* and one strain of *Streptococcus pyogenes*. Both were clinical isolates. Bacterial cells were counted and adjusted to the number of organisms which would give $50 \sim 200$ LD_{50}s.in a 0.5-ml dose. The bacteria were grown in brain-heart infusion (Difco Laboratories, Detroit, MI.) and the inoculum adjusted to the proper concentration with brain-heart infusion or brain-heart infusion with 5.0% mucin. The mice used were $19 \sim 21$ g random sex ICR mice (Harlan Industries, Cumberland, IN.). Mice (8 per group) were infected intraperitoneally (ip) with 0.5 ml of the bacterial suspension. Antibiotic was administered subcutaneously at 1 and 5 hours post-infection. The animals were observed for 7 days post-infection. A control group of mice (five dilutions with 8 mice per dilution) was included with the test animals to titrate the LD_{50} of the infecting organism. An ED_{50} was calculated at 7 days after infection by the method of REED and MUENCH[8].

The LD_{50} determinations were done in $19 \sim 21$ g random sex ICR mice. Mice (4 per group) were injected by the intraperitoneal route with the appropriate 2-fold dilution of the antibiotic. Each dilution was injected into a different group. These mice were then observed for 7 days. Deaths in each group were recorded daily. The LD_{50} was calculated according to the method of REED and MUENCH[8].

Methods for the Determination of Effect on Macromolecular Biosynthesis

Radioactivity from incorporation of [^{14}C]leucine, [^{14}C]thymidine, [^{14}C]uracil, and [^{14}C]alanine into trichloroacetic acid insoluble protein, DNA, RNA, and cell wall peptidoglycan, respectively, of *S. aureus* FDA 209P (ATCC 6538P) was measured exactly as described previously[9]. Test compounds were dissolved in aqueous DMSO. Final concentration of solvent was 1%, which gave less than 3% inhibition in any of the assays.

Results and Discussion

Taxonomy

Taxonomic studies identified the strain A80915, as *S. aculeolatus*[5]. Hydrolyzed whole cells contained LL-diaminopimelic acid. Three diagnostic sugars; galactose, glucose, and ribose; were found in whole cell hydrolysates. Thus, strain A80915 has a type I cell wall and a non characteristic sugar pattern[10]. The presence of these components is consistent with the genus *Streptomyces*. The strain A80915 is very similar to the SF2415-producing culture, *S. aculeolatus*[5]. A taxonomic literature comparison of these two cultures; which differed only in temperature range, NaCl tolerance, and the ability to reduce nitrates; is summarized in Table 1. These differences were considered to be strain, not species, differences. Scanning electron micrographs of strain A80915 are shown in Fig. 1. The strain A80915 was also compared to the napyradiomycin-producing culture *Chainia rubra* MG802-AF1[1] and the tetronomycin-producing culture *Streptomyces* sp. S 53161/A[7]. Sclerotia, characteristic of the genus *Chainia*, were not observed in strain A80915. The tetronomycin-producing culture was not as closely related by morphological or physiological characteristics to strain A80915 as *S. aculeolatus*. The strain A80915 was deposited to the ARS Culture Collection, Northern Regional Research Center, under the accession No. NRRL 18422.

† National Committee for Clinical Laboratory Standards. Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A. National Committee for Clinical Laboratory Standards, Villanova, 1985.

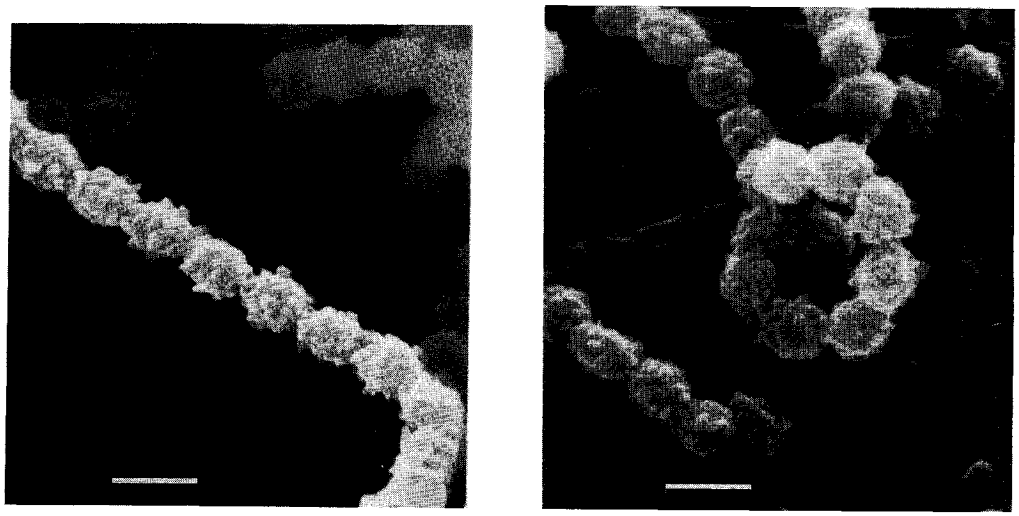
Table 1. Taxonomic comparison of similarities and differences between strain A80915 and *Streptomyces aculeolatus*[5].

Characteristic	Strain A80915	*S. aculeolatus*
Aerial color	White (Yellow)	White (Yellow)
Reverse color	Brown to Orange	Brown to Orange
Soluble pigment	reddish brown	reddish brown
Morphology	RA (S)	RA (S)
Temperature range (°C)	20~30	15~37
NaCl tolerance (%)	5	3~4
Gelatin liquefaction	+	+
Melanoid pigments:		
ISP1	−	−
ISP6	−	−
ISP7	−	−
Nitrate reduction	+	−
Starch hydrolysis	+	+
Peptonization of milk	−	−
Carbohydrate utilization:		
L-Arabinose	+	+
Fructose	+	+
Glucose	+	+
Inositol	−	−
Mannitol	+	+
Raffinose	+	+
L-Rhamnose	+	+
Sucrose	−	−
Xylose	+	+
Formation of sclerotia	−	−
Spore surface	Warty	Warty
Spore shape	Spherical	Ellipsoidal

RA: *Retinaculum-Apertum*, S: *Spira*.

Fig. 1. Scanning electron micrographs of strain A80915 illustrating the warty surface ornamentation and spherical spore shape.

Bar equals 1.0 mm.



The culture was grown on tap water agar for 12 days at 30°C.

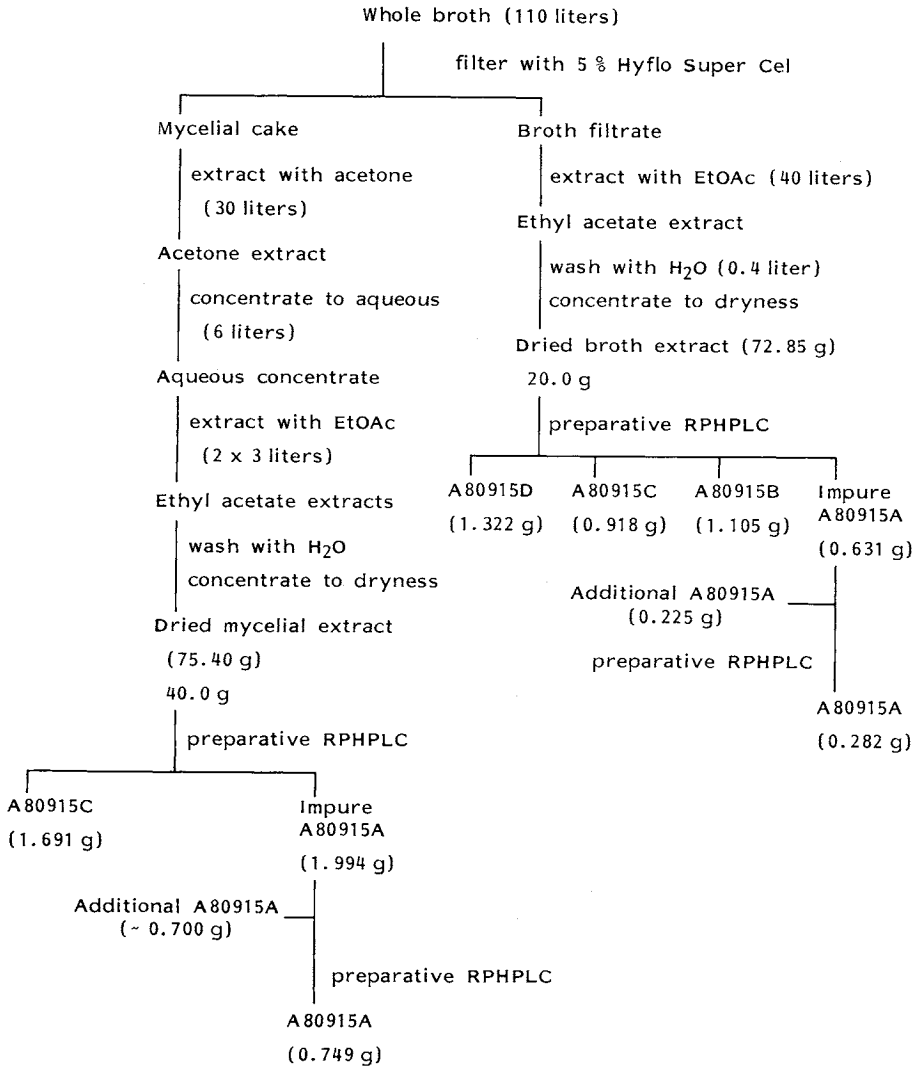
Fermentation

The strain A80915 was grown in ATCC 172 vegetative medium for 48～72 hours at 30°C and this vegetative culture was used to inoculate the M1 and M2 fermentation media described in Table 2. In the

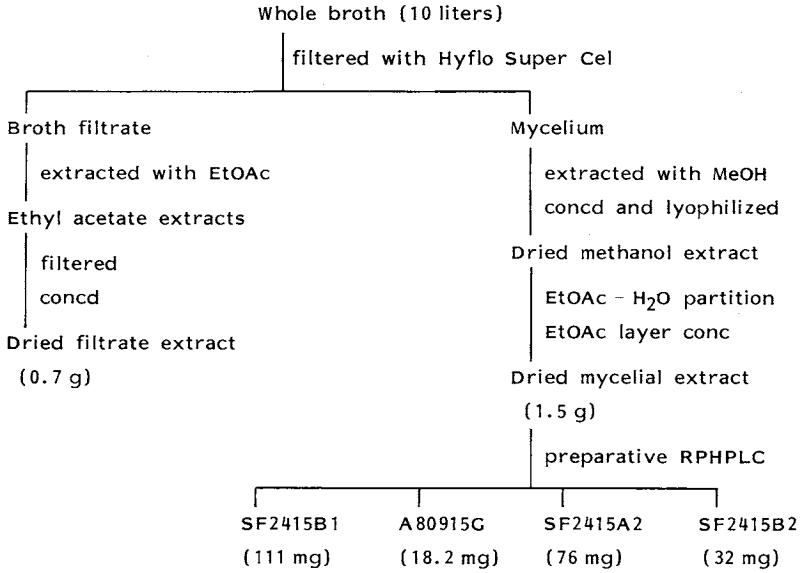
Table 2. A80915 fermentation media M1 and M2.

M1	%	M2	%
Glucose	1.0	Soybean flour	0.6
Blackstrap molasses	2.0	Potato dextrin	3.0
Bacto-peptone	0.5	Sodium nitrate	0.1
Calcium carbonate	0.2	Calcium carbonate	0.4
KCl	0.02	$MgSO_4 \cdot 7H_2O$	0.0004
$MgSO_4 \cdot 7H_2O$	0.02	$FeSO_4 \cdot 7H_2O$	0.0004
$FeSO_4 \cdot 7H_2O$	0.0004	pH adjusted to	7.0
pH adjusted to	7.0		

Scheme 1. Isolation for A80915 factors A～D produced in medium M1.



Scheme 2. Isolation for A80915 factor G produced in medium M2.



M1 medium, four novel factors designated A80915A, B, C, and D, and napyradiomycin B1 were normally produced. Addition of dibasic potassium phosphate to the M1 medium inhibited A80915 factors, suggesting a regulatory role of phosphate in A80915 biosynthesis. Further evidence for phosphate regulation was acquired when phosphate addition (0.1%) to the M2 medium produced primarily tetronomycin (data not shown). Deletion of phosphate from the M2 medium then resulted in biosynthesis of the SF2415 antibiotics with no apparent synthesis of the polyether or A80915 factors A, B, C, and D.

It seems clear that phosphate can effectively regulate the biosynthesis of A80915 and related antibiotics. It should be pointed out that the phosphate concentration is about 4- to 8-fold higher in M1 than M2 (Table 2). In addition, the medium makeup apparently plays a vital role in the selective biosynthesis of the novel A80915 complex, SF2415 factors, tetronomycin, and napyradiomycins. For example, preliminary studies in modified M1 and M2 (replacing the original nitrogen source with another) have resulted in the biosynthesis of additional A80915 factors (NAKATSUKASA and MABE, unpublished observations). These results were not surprising since fermentation conditions (temperature, nutrition, *etc.*) can profoundly affect biosynthesis of microbial metabolites. The strain A80915 is an excellent example of the capacity of one organism to synthesize products formerly produced by three distinct microorganisms; napyradiomycin by *C. rubra*, tetronomycin by *Streptomyces* sp. S53161/A and SF2415 factors by *S. aculeolatus*. It is indeed tempting to speculate that these three cultures may possess traits similar to strain A80915 when they are grown under similar fermentation conditions.

Isolation and Characterization of A80915 Factors

The isolation of A80915 factors A ~ D from the fermentation of *S. aculeolatus* NRRL 18422 in medium M1 is summarized in Scheme 1. Factors A and C, present in both the broth filtrate and the mycelial methanol extract, and factors B and D, found predominantly in the broth filtrate, were recovered by extraction with ethyl acetate. Purification of A80915A ~ D and the coproduced napyradiomycin B1[1,2] was accomplished by preparative RPHPLC on C_{18} bonded silica supports. The isolation of A80915G from the mycelial extract of *S. aculeolatus* NRRL 18422 in medium M2 is shown in Scheme 2.

Again, purification of A80915G as well as the coproduced SF2415 factors A2, B1, and B2[5,6] was accomplished by preparative RPHPLC of the EtOAc extract. HPLC retention times of A80915 factors and coproduced, previously reported semi-naphthaquinone antibiotics are listed in Table 3.

Physico-chemical Properties

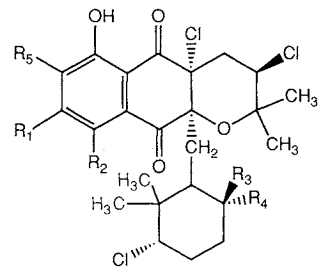
The A80915 antibiotics are soluble in chloroform, ethyl acetate, methanol, and dimethyl sulfoxide; but are insoluble in hexane and water. The physico-chemical characteristics of the A80915 factors are listed in Table 4. Factors A and C are related to factors B and D, respectively, through the loss of N_2 from the molecular formulae. This is consistent with the presence of diazo group absorptions in the IR spectra of factors B and D, but not A and C. The structure elucidation of A80915 factors (Figs. 2 and 3) will be described elsewhere.

Table 3. HPLC Rt's for semi-naphthaquinone antibiotics isolated from strain A80915.

Compound	Rt (minutes)
A80915A	10.4
A80915B	7.1
A80915C	5.2
A80915D	3.7
A80915G	6.9
Napyradiomycin B1	8.2
SF2415A2	6.5
SF2415B1	4.6
SF2415B2	8.6

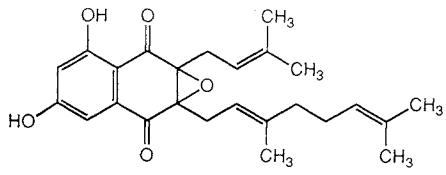
HPLC system as described in Materials and Methods section.

Fig. 2. Structures of semi-naphthaquinone antibiotics isolated from strain A80915 fermented in medium M1 and related napyradiomycin B4[3].

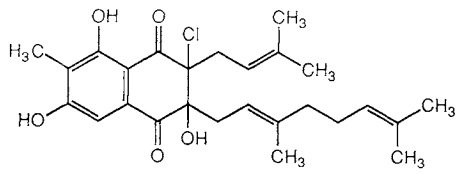


	R_1	R_2	R_3	R_4	R_5
A80915A	OH	H	$=CH_2$	—	CH_3
A80915B	O^-	$N\equiv N^+$	$=CH_2$	—	CH_3
A80915C	OH	H	OH	CH_3	CH_3
A80915D	O^-	$N\equiv N^+$	OH	CH_3	CH_3
Napyradiomycin B_1	OH	H	$=CH_2$	—	H
Napyradiomycin B_4	OH	H	OH	CH_3	H

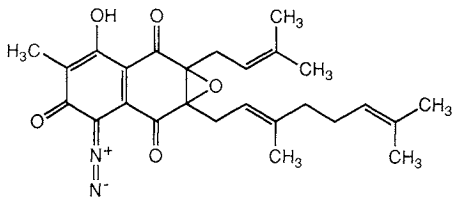
Fig. 3. Structures of semi-naphthaquinone antibiotics isolated from strain A80915 fermented in medium M2.



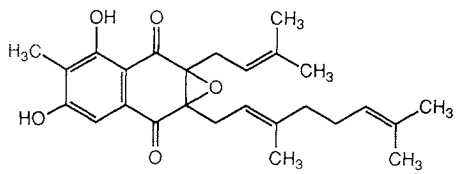
A80915G



SF2415B1



SF2415A2



SF2415B2

Table 4. Physico-chemical properties of A80915 factors.

	A80915A	A80915B	A80915C	A80915D
Appearance	Pale yellow amorphous powder	Red oil	Yellow prisms	Red amorphous powder
Molecular formula	$C_{26}H_{31}Cl_3O_5$	$C_{26}H_{29}Cl_3N_2O_5$	$C_{26}H_{33}Cl_3O_6$	$C_{26}H_{31}Cl_3N_2O_6$
MS				
HR-MS (M+H) Calcd:	529.13152	555.12201	547.14208	573.13258
Found:	529.12891 (FAB-MS)	555.12045 (FAB-MS)	547.14478 (FAB-MS)	573.1326 (FAB-MS)
UV λ_{max}^{EtOH} nm (ε)	266 (17,330), 332 (7,350), 358 (sh, 7,040)	259 (12,460), 301 (10,320), 366 (4,680)	267 (19,490), 314 (9,430), 356 (7,890)	261 (16,680), 305 (15,170), 403 (4,860), 482 (1,950)
$\lambda_{max}^{EtOH-HCl}$ nm (ε)	266 (17,360), 330 (7,320), 358 (sh, 7,040)	258 (12,820), 301 (9,940), 361 (3,920), 435 (1,660)	266 (22,550), 331 (8,860), 356 (sh, 8,270)	257 (13,400), 302 (10,200), 365 (4,450), 431 (2,070)
$\lambda_{max}^{EtOH-NaOH}$ nm (ε)	246 (13,500), 300 (15,090), 401 (13,490)	265 (15,300), 306 (14,330), 395 (7,780)	246 (15,450), 304 (21,060), 400 (15,220)	265 (sh, 19,620), 306 (sh, 17,870), 402 (10,060)
IR (KBr) cm^{-1}	3406, 2976, 2936, 1701, 1636, 1604, 1371, 1348, 1301, 1282, 1120, 1094, 1083, 885, 760	3410, 2980, 2940, 2159, 2140, 1700, 1649, 1614, 1592, 1510, 1428, 1388, 1369, 1162, 1111, 1098	3376, 2976, 2936, 1701, 1636, 1604, 1349, 1323, 1284, 1118, 1086, 1058, 885, 846, 760	3490, 2981, 2950, 2163, 2144, 1692, 1651, 1613, 1510, 1429, 1388, 1366, 1163, 1089
$[\alpha]_{589}^{25}$	−89.7° (*c* 1.00, MeOH)	−22.2° (*c* 1.04, MeOH)	−115.4° (*c* 0.56, MeOH)	−79.2° (*c* 1.06, MeOH)

	A80915G
Appearance	Reddish-brown oil
Molecular formula	$C_{25}H_{30}O_5$
MS	
HR-MS (M+H) Calcd:	411.2171
Found:	411.2171 (FAB-MS)
UV λ_{max}^{EtOH} nm (ε)	253 (11,000), 301 (6,720), 368 (5,240)
$\lambda_{max}^{EtOH-HCl}$ nm (ε)	253 (12,600), 309 (4,040), 360 (5,210)
$\lambda_{max}^{EtOH-NaOH}$ nm (ε)	294 (28,200), 391 (17,300)
IR ($CHCl_3$) cm^{-1}	1697, 1637, 1618

Antimicrobial Evaluation

In Vitro Antimicrobial Activity

The *in vitro* activity of A80915 factors is presented in Tables 5 and 6. The antibacterial spectrum is mostly limited to Gram-positive microorganisms, although some anaerobic as well as aerobic Gram-negative

Table 5. *In vitro* activity of A80915 factors against aerobic bacteria.

Organism	MIC (μg/ml)				
	A80915A	A80915B	A80915C	A80915D	A80915G
Staphylococcus aureus X1.1	1	0.03	>128	0.25	8
S. aureus V41	1	0.06	>128	0.25	4
S. aureus X400	0.5	0.125	>128	0.5	8
S. aureus S13E	1	0.06	>128	0.25	4
S. epidermidis 270	0.5	0.06	>128	0.25	16
S. epidermidis 222	0.25	0.015	>128	0.125	2
Streptococcus pyogenes C203	0.25	0.03	64	0.125	2
S. pneumoniae PARK	0.5	0.125	>128	0.125	2
Enterococcus faecium X66	2	1	>128	8	4
E. faecalis 2041	1	1	>128	8	8
Haemophilus influenzae C. L.	<0.008	<0.008	>128	8	>128
H. influenzae 76	<0.008	<0.008	>128	8	>128
Escherichia coli N10	>128	>128	>128	>128	>128
Klebsiella pneumoniae X26	>128	>128	>128	>128	>128
Enterobacter aerogenes C32	>128	>128	>128	>128	>128
E. cloacae EB5	>128	>128	>128	>128	>128
Salmonella sp. X514	>128	>128	>128	>128	>128
Pseudomonas sp. X528	>128	>128	>128	>128	>128
Serratia marcescens X99	>128	>128	>128	>128	>128
Shigella sonnei N9	>128	>128	>128	>128	>128
Proteus rettgeri C24	>128	>128	>128	>128	>128
Citrobacter freundii CF17	>128	>128	>128	>128	>128
Acinetobacter calcoaceticus AC12	>128	>128	>128	>128	>128

Table 6. *In vitro* activity of A80915 factors against anaerobic bacteria.

Organism	MIC (μg/ml)				
	A80915A	A80915B	A80915C	A80915D	A80915G
Clostridium difficile 2994	2	2	16	4	4
C. perfringens 81	2	2	16	4	4
C. septicum 1128	2	1	16	2	1
Eubacterium aerofaciens 1235	2	2	16	2	0.5
Peptococcus asaccharolyticus 1302	4	2	16	4	0.5
P. prevotii 1281	2	1	16	2	8
Peptostreptococcus anaerobius 1428	4	2	16	2	2
P. intermedius 1264	2	1	16	2	2
Propionibacterium acnes 79	1	0.5	8	1	0.5
Bacteroides fragillis 111	128	8	128	8	64
B. fragillis 1877	16	4	16	2	8
B. fragillis 1936B	32	4	16	2	8
B. thetaiotaomicron 1438	>128	128	>128	8	>128
B. melaninogenicus 1856/28	2	1	16	2	0.5
B. melaninogenicus 2736	128	64	16	2	16
B. vulgatus 1211	32	4	>128	8	16
B. corrodens 1874	4	2	16	2	8
Fusobacterium symbiosum 1470	4	2	>128	2	0.5
F. necrophorum 6054A	>128	>128	>128	>128	>128

strains were sensitive.

Acute Toxicity

As shown in Table 7, A80915 factors B and D were toxic to mice when given by the ip route (LD_{50} values of 75 and 11.8 mg/kg, respectively). Factors A and C, which lack the diazo substituent, are much less toxic, with LD_{50} values of 212 and >300 mg/kg, respectively.

Efficacy Studies

Based upon the *in vitro* antimicrobial potencies and acute toxicities, A80915A was selected for *in vivo* efficacy studies. Unfortunately, A80915A was found to be nonefficacious against *S. aureus* ($ED_{50}>20$ mg/kg $\times$ 2) and *S. pyogenes* ($ED_{50}>50$ mg/kg $\times$ 2) systemic infection models in mice when administered by the subcutaneous route.

Studies of Effect on Macromolecular Biosynthesis

The effects of A80915 factors A, B, C, and D on macromolecular biosynthesis were measured in intact cells during a 10 to 15 minutes incubation to insure detection of initial effects on cell metabolism[9]. Under these conditions, all factors showed a rapid and potent inhibition of protein, DNA, RNA, and cell wall biosynthesis (Table 8). Similar results on Gram-positive bacteria have been reported with a 1,4-naphthaquinone derivative[11]. The generalized inhibition caused by A80915 antibiotics may be due to effects on events associated with the bacterial cytoplasmic membrane.

In summary, five new seminaphthaquinone antibiotics have been isolated from strain A80915 (*S. aculeolatus* NRRL 18422). Four of the new antibiotics, A80915A~D, which are produced in medium M1, are structurally related to the napyradiomycins, although diazo-containing napyradiomycins have not previously been reported. Factor A80915G, which is produced in medium M2, is structurally related to SF2415B2. The new A80915 factors show antimicrobial activity against Gram-positive bacteria *in vitro* through inhibition of protein, DNA, RNA, and cell wall biosynthesis. Factor A80915A, which shows good antibacterial MICs with minimal *in vivo* toxicity, did not protect mice against systemic infections of *S. aureus* or *S. pyogenes*.

Table 7. Acute toxicity of A80915 antibiotics in mice.

Compound	LD_{50} (mg/kg, ip)
A80915A	212
A80915B	75
A80915C	>300
A80915D	12

Table 8. Effects of A80915 factors on macromolecular biosynthesis in *Staphylococcus aureus*[a].

Antibiotic	% Inhibition at antibiotic concentration[b]											
	Protein			DNA			RNA			Cell wall		
	100	10	1	100	10	1	100	10	1	100	10	1
A80915A	100	90	85	99	91	62	100	99	99	100	98	98
A80915B	100	100	93	96	95	54	100	100	95	99	99	85
A80915C	94	99	96	94	94	74	100	100	100	98	94	48
A80915D	100	100	70	98	97	23	100	98	92	99	93	31

[a] Macromolecular biosynthesis was measured as described in Materials and Methods.
[b] Compounds were tested at 100, 10, and 1 μg/ml.

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References

1) SHIOMI, K.; H. IINUMA, M. HAMADA, H. NAGANAWA, M. MANABE, C. MATSUKI, T. TAKEUCHI & H. UMEZAWA: Novel antibiotics napyradiomycins. Production, isolation, physico-chemical properties and biological activity. J. Antibiotics 39: 487~493, 1986
2) SHIOMI, K.; H. NAKAMURA, H. IINUMA, H. NAGANAWA, K. ISSHIKI, T. TAKEUCHI, H. UMEZAWA & Y. IITAKA: Structures of new antibiotics napyradiomycins. J. Antibiotics 39: 494~501, 1986
3) SHIOMI, K.; H. NAKAMURA, H, IINUMA, H. NAGANAWA, T. TAKEUCHI, H. UMEZAWA & Y. IITAKA: New antibiotic napyradiomycins A2 and B4 and stereochemistry of napyradiomycins. J. Antibiotics 40: 1213~1219, 1987
4) SHIOMI, K.; H. IINUMA, H. NAGANAWA, K. ISSHIKI, T. TAKEUCHI & H. UMEZAWA: Biosynthesis of napyradiomycins. J. Antibiotics 40: 1740~1745, 1987
5) SHOMURA, T.; S. GOMI, M. ITO, J. YOSHIDA, E. TANAKA, S. AMANO, H. WATABE, S. OHUCHI, J. ITOH, M. SEZAKI, H. TAKEBE & K. UOTANI: Studies on new antibiotics SF2415. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activities. J. Antibiotics 40: 732~739, 1987
6) GOMI, S.; S. OHUCHI, T. SASAKI, J. ITOH & M. SEZAKI: Studies on new antibiotics SF2415. II. The structural elucidation. J. Antibiotics 40: 740~749, 1987
7) KELLER-JUSLÉN, C.; H. D. KING, M. KUHN, H.-R. LOOSLI, W. PACHE, T. J. PETCHER, H. P. WEBER & A. VON WARTBURG: Tetronomycin, a novel polyether of unusual structure. J. Antibiotics 35: 142~150, 1982
8) REED, L. J. & H. MUENCH: A simple method for estimating fifty percent endpoints. Am. J. Hygiene 27: 493~497, 1938
9) ALLEN, N. E.; J. N. HOBBS, Jr. & W. E. ALBORN, Jr.: Inhibition of peptidoglycan biosynthesis in gram-positive bacteria by LY146032. Antimicrob. Agents Chemother. 31: 1093~1099, 1987
10) LECHEVALIER, M. P. & H. LECHEVALIER: Chemical composition as a criterion in the classification of aerobic actinomycetes. Int. J. Syst. Bacteriol. 20: 435~443, 1970
11) OLENICK, J. G. & F. E. HAHN: Bactericidal action of a 2-hydroxy-3-alkyl-1,4-naphthoquinone: Blockade of metabolite permeation across the membrane. Ann. N. Y. Acad. Sci. 235: 542~552, 1974