ANTIFUNGAL ANTHRACYCLINE ANTIBIOTICS, SPARTANAMICINS A AND B FROM *Micromonospora* spp.†

MURALEEDHARAN G. NAIR, SAROJ K. MISHRA†† and ALAN R. PUTNAM†††

Bioactive Natural Products Laboratory, Department of Horticulture,
Michigan State University,
East Lansing, Michigan 48824, U.S.A.

RAMESH C. PANDEY

XeChem, Inc.,
100 Jersey Avenue, New Brunswick,
New Jersey 08901-3279, U.S.A.

(Received for publication August 24, 1992)

Spartanamicins A and B, two antifungal antibiotics, were produced by a culture of *Micromonospora* spp. strain No. MSU-43097 (ATCC 53803), isolated from a potted soil containing asparagus (*Asparagus officinalis* L.) plants. The antibiotics were isolated from the mycelial cake using organic solvents. The structures of spartanamicins A and B were determined by spectral and chemical means. Spartanamicin B is more active as an antifungal compound than it's analogue, A. The minimum inhibitory concentration for spartanamicin B on *Candida albicans* and *Aspergillus*, *Cladosporium*, *Cryptococcus*, *Rhodotorula* and *Staphylococcus* spp. ranged from 0.2 to 1 μg/ml. It was not active against *Staphylococcus aureus*, *Escherichia coli* and *Citrobacter* spp. but some strains of *S. aureus* were sensitive.

Micromonospora species are well known for their ability to produce biologically active compounds. Calicheamicin, approximately 1,000-fold more active than doxorubicin against murine tumors, is an unusually powerful antitumor compound isolated from *Micromonospora* subsp. *echinospora calichenis*[1~3]. Triglycosidic antibiotics, isolated from *M. inositola* and *M. megalomicea* and other antibiotics reported from *Micromonospora* species are chalcidin complex, everninomicins B and D[4,5] and ferrioxamine B[6,7]. *Streptomyces galilaeus* produces 21 anthracycline antibiotics out of which aclacinomycins A, B, and their derivatives were studied for their antitumor activities[8~14]. We report the isolation, purification, and identification of potent antifungal compounds belonging to the anthracycline group of antibiotics.

Results and Discussion

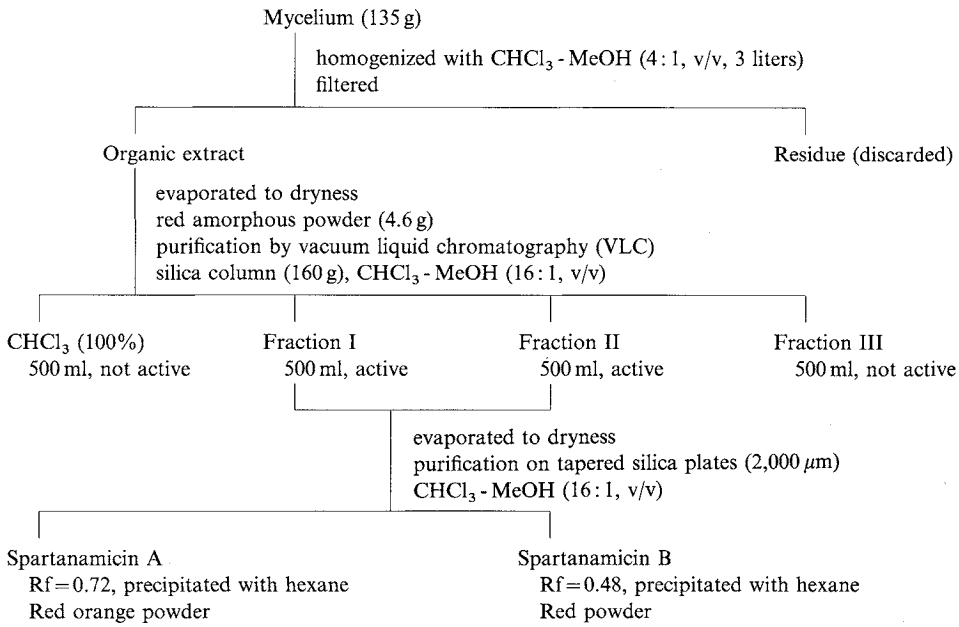
The strain 43097 is relatively easy to grow in suspension and solid agar plates. Preliminaly taxonomic studies indicated that this organism is a *Micromonospora* spp. It is deep red in color on solid YMG plate at 7~8 days and orange at 3~4 days. It also grew well in all the 9 different media studied but produced spartanamicins A and B in YMG medium only. The pH of the fermentation medium remained virtually unchanged for 12 days and the mycelium can be harvested for spartanamicins A and B between days 3~4.

† US Patents 4,977,084; 5,013,549; 5,066,585.

†† Current address: 919 Sunrise Knollway, Houston, TX 77062, U.S.A.

††† Current address: 49823 Gallatin Road, Gallatin Gateway, Montanna 59730, U.S.A.

Fig. 1. Isolation and purification of spartanamicins A and B from the mycelial cake of the strain 43097.



Spartanamicins A and B are extracted from the mycelial cake of the strain 43097 using organic solvent mixtures (Fig. 1). No antibiotic was detected in the cell free fermentation broth. The crude extract was analyzed by TLC plates and after marking the respective spots, they were incubated with *Candida albicans*, *Aspergillus fumigatus* and *Cladosporium* spp. Only two zones of inhibition were seen on the plate. The high Rf compound was less active than the lower Rf one. Further analysis of these active bands indicated that these compounds as spartanamicins A and B, for the high and low Rf, respectively. Since spartanamicin B was found to be much more active than it's analogue A, further biological activity was carried out only on spartanamicin B.

For MIC determination of spartanamicin B on fungi and bacteria, it was dissolved in DMSO to obtain a stock solution. This solution (20 μl) was mixed with 2 ml of liquid EMMON's medium containing *ca.* 10,000 cfu of fungal test strains or 100,000 cfu of bacterial test strains (Table 1). MIC values for *Cladosporium* spp. and *A. fumigatus* were 0.4 and 0.6 μg/ml for other *Aspergillus* spp. (Table 1). Most importantly, *C. albicans* was inhibited at 0.2 μg/ml and the same was for *Penicillium* spp. The MIC values for other fungi and bacteria are listed in Table 1. It is also important to note that *Citrobacter* spp., *Escherichia coli*, and multiple drug-resistant *Staphylococcus aureus* were not affected by spartanamicin B. Since spartanamicin B showed an MIC value of 0.2 μg/ml for *C. albicans*, it can be considered as a strong candidate to substitute amphotericin B for the treatment of *C. albicans*.

These compounds are in the same class of anthracycline antibiotics isolated from *Streptomyces galilaeus*[10~13]. Spartanamicin B was also found to be similar to 1-hydroxy MA144M1 produced by *S. galilaeus*[10,12]. Both A and B forms in cinerubin and aclacinomycin showed differences only in their terminal sugars[15]. Cinerubins and aclacinomycins differed in their aglycone moiety.

Isolation and purification of spartanamicins A and B are illustrated in Fig. 1. Processing the mycelium from 10 liters of fermentation broth (Fig. 1) afforded analytically pure spartanamicin B (450 mg) and

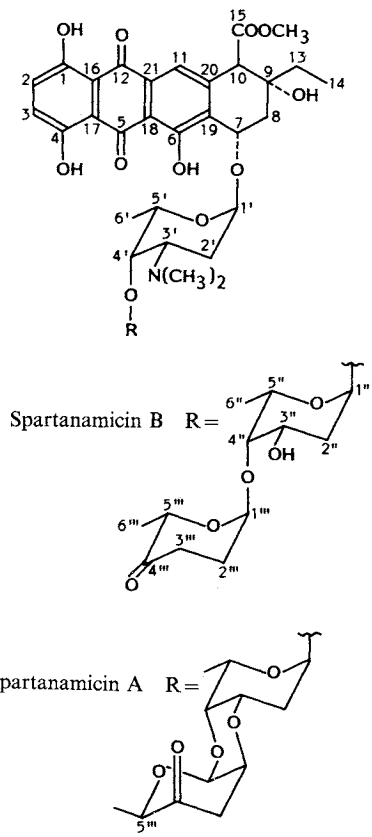
Table 1. Minimum inhibitory concentration (MIC) of spartanamicin B against certain fungi and bacteria.

Organisms	MIC (μg/ml)	Organisms	MIC (μg/ml)
Aspergillus fumigatus	0.4	*C. neoformans* serotype-D	0.8
A. niger	0.6	*Rhodotorula rubra*	0.8
A. flavus	0.6	*R. glutinis*	0.4
Penicillium spp.	0.2	*Citrobacter* spp.[a]	Not active
Cladosporium spp.	0.4	*Pseudomonas aeruginosa*[a]	100
Candida albicans	0.2	*Klebsiella pneumoniae*[a]	100
C. quilliermondii	0.8	*Escherichia coli*[a]	Not active
Cryptococcus neoformans-N-2	0.8	*Staphylococcus aureus* ATCC 29213[a]	0.8
C. neoformans-N-3	0.6	*S. aureus* ATCC 25923[a]	0.8
C. neoformans-G-3	1.0	*S. aureus* (multiple drug resistant)[a]	Not active
C. neoformans serotype-C	0.8		

N: Encapsulated, mucoid strains with neurotropic tendencies.
G: Non-encapsulated, dry-pasty, highly virulent strain.
[a] Bacterial strains; all others are fungi.

spartanamicin A (250 mg). Acid hydrolysis of spartanamicins A and B afforded the same aglycone, spartanone. The structure of this aglycone is confirmed to be as shown in Fig. 2 and is identical to pyrromycinone[8~14]. Mass spectral analysis of the aglycone (by FAB and CI) did not give a molecular ion at *m/z* 428 due to the aromatization of the aliphatic ring under MS condition. ^{1}H NMR spectrum of spartanone indicated that there was no dehydration during the work up of the hydrolysis reaction.

Fig. 2. Structures of spartanamicins A and B.



Proton correlation spectroscopy of spartanamicin B gave the correlation of the single aromatic proton at δ 4.11 (H-10) to H-11 at δ 7.69. This proton was also coupled to one of the C-8 protons appeared as the dd (Table 2) at 2.35 ppm. There was no other coupling for this proton as evident from the COSY spectrum. Spartanamicin A also showed an identical H-10 to H-11 and H-10 to H-8 correlation.

Comparison of the ^{1}H and ^{13}C NMR spectra of both spartanamicins suggested a 22-carbon aglycone and 3 sugar residues with 20 carbons (Tables 2 and 3). All three sugar residues contained a 5-*C*-methyl group each. The signal at 210 ppm, for an aliphatic ketone, and the *N*-methyl groups appeared at 43.21 and 43.28 ppm, respectively, in spartanamicins A and B are assigned to the rhodosamine sugar moieties. Collision activated dissociation (CAD)-MS of spartanamicin B gave daughter ions at *m/z* 698,

Table 2. ^{1}H NMR chemical shift assignments of spartanamicins A and B.

Position	Spartanamicin B		Spartanamicin A	
	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
1	12.92 s		12.94 s	
2	7.27 d	9.4	7.29 d	9.5
3	7.29 d	9.4	7.31 d	9.5
4	12.76 s		12.78 s	
6	12.17 s		12.20 s	
7	5.30 br d	4.2	5.25 br d	4.2
8ax	2.40 d	15.0	2.45 dd	15.0/4.2
8eq	2.35 dd	15.0/4.2	2.53 dd	15.0/4.2
10	4.11 s		4.15 s	
11	7.69 s		7.70 s	
13a	1.51 m		1.50 m	
13b	1.75 m		1.72 m	
14	1.15 t	7.3	1.10 t	7.0
OCH_3	3.69 s		3.70 s	
1′	5.56 br s		5.50 br s	
2′ax	1.81 m		1.85 m	
2′eq	1.95 m		1.95 dd	15.0/4.0
3′	3.78 m		3.75 m	
4′	3.62 br s		3.77 br s	
5′	4.54 q	6.2	4.80 q	6.8
6′	1.34 d	6.4	1.29 t	7.0
$N(CH_3)_2$	2.20 s		2.6 s	
1″	5.08 d	3.5	5.0 d	3.0
2″ax	2.10 m		2.5 m	
2″eq	2.15 m		2.0 m	
3″	4.16 m		4.8 m	
4″	3.76 s		4.5 s	
5″	4.19 q	6.8	4.0 q	6.4
6″	1.22 d	6.4	1.2 d	7.0
1‴	5.12 t	6.4	5.0 d	3.0
2‴ax	2.16 m		4.0 t	4.0
2‴eq, 3‴ax, eq	2.40～2.60 m		4.0～4.01 m	
5‴	4.62 q	6.4	4.5 q	6.4
6‴	1.38 d	6.7	1.8 d	6.4

586, 393, 349 and 335. The ion at m/z 586, analyzed for $C_{30}H_{34}O_{12}$, was common for both spartanamicins and assigned to a fragment consisting the aglycone with rhodosamine sugar residue. The unique fragment at m/z 698 in spartanamicin B CAD-MS was produced by the loss of the terminal cinerulose sugar moiety. This fragment was absent in spartanamicin A and indicated the nature of the terminal sugar linkage (Fig. 2) for this compound. Mass spectral evidence of the common fragment at m/z 586 in both spartanamicins and lack of fragment at m/z 698 by spartanamicin A were in agreement with the ^{1}H and ^{13}C NMR data and confirmed the proposed structure in Fig. 2 for these compounds.

Acetylation of spartanamicin B afforded tri-acetate of the aglycone and mixtures of acetates of the natural product. The crude product from the acid hydrolysis of spartanamicin B was acetylated and analyzed by GC-MS. The sugar residues were thus characterized as rhodosamine, deoxy-fucose and cinerulose. A comparison of the NMR, MS and GC-MS of the hydrolyzed sugar portions of spartanamicins A and B indicated that their structures are as shown in Fig. 2.

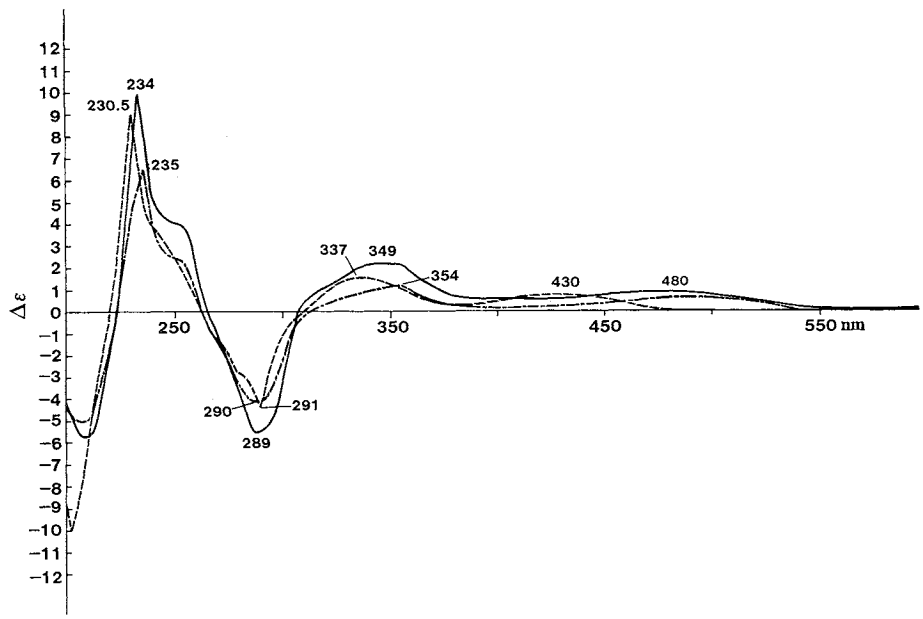
It is important to note that spartanamicins A and B were not soluble in MeOH or EtOH while related

Table 3. ^{13}C NMR chemical shift assignments of spartanamicins A and B in $CDCl_3$.

Position	Spartanamicin A	Spartanamicin B	Position	Spartanamicin A	Spartanamicin B
1	157.83	157.77	OCH_8	52.54	52.53
2	129.73	157.77	1′	101.56	101.66
3	130.09	130.05	2′	29.27	29.22
4	158.43	158.41	3′	61.50	61.60
5	190.62	190.48	4′	74.13	74.03
6	162.28	162.27	5′	70.64	70.66
7	65.28	65.33	6′	17.88	16.95
8	33.75	34.32	$N(CH_3)_2$	43.21	43.28
9	71.67	71.78	1″	99.07	100.14
10	57.16	57.15	2″	26.98	33.79
11	120.40	120.36	3″	67.27	68.35
12	185.77	185.63	4″	68.28	82.94
13	32.17	32.18	5″	66.91	71.60
14	6.67	6.71	6″	16.03	17.85
15	171.32	171.27	1‴	91.54	99.42
16	112.52	112.43	2‴	62.98	27.66
17	112.36	112.27	3‴	39.73	33.52
18	114.81	114.71	4‴	208.25	210.11
19	131.49	131.52	5‴	77.93	71.72
20	142.45	142.44	6‴	16.18	14.80
21	132.80	132.68			

Fig. 3. CD curves of spartanamicins A and B and aclacinomycin.

—— Spartanamicin A, —·— spartanamicin B, – – – aclacinomycin A.



anthracycline antibiotics, cinerubins and aclacinomycins, were readily soluble in alcohols. A comparative CD spectra (Fig. 3) of an equimolar quantities of aclacinomycin, spartanamicins A and B revealed that all three of them have a similar CD pattern with difference in their $\Delta\varepsilon$ values at varying λ. Antifungal activity

of spartanamicins, especially the B analogue, are also significantly higher than the reported anthracycline antibiotics. It is possible that spartanamicin B with potent antifungal activity and aclacinomycins and related anthracycline antibiotics with marginal antifungal activity might have different configurations in their sugar moieties and hence the difference in their solubilities and antifungal activities. This is the first report of anthracycline antibiotics of this nature produced by a *Micromonospora* spp.

Experimental

Melting points are uncorrected and determined on a Kofler hot stage apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker 300, Varian Gemini 300 and GE Omega 500 spectrometers using $CDCl_3$ as solvent. FAB-MS were obtained by bombarding glycerol solutions of spartanamicins with 4~6 keV xenon atoms. Collison activated dissociation spectra (CAD) was obtained by bombarding parent ions with He molecules. IR and UV spectra were recorded on a Perkin-Elmer model 1170 FT-IR spectrometer and Gilford Response II UV spectrometer, respectively. CD spectra were recorded on a JASCO model 500 spectrometer and are for MeOH solutions. HPLC analyses of spartanamicin complex was carried out on Novapak silica column (3.9×160 mm) using $CHCl_3$-MeOH-HCOOH (50:48:2) mobile phase at 1.5 ml/minute. The detection was at 490 nm using a diode array detector.

Isolation of the Strain 43097

The strain 43097 was isolated from a soil sample collected from the rhizosphere of an indoor potted asparagus plant. One g of the soil was vortexed with 9 ml of physiological saline (0.85% NaCl in distilled water) and serial dilutions prepared. A 0.1 ml of the suspension was uniformly spread on the surface of NZ-Amine A agar (NZ-Amine A 3 g, Bacto agar 18 g, tap water 1 liter) fortified with nystatin (0.03 g/liter) and cycloheximide (0.5 g/liter) in sterile plastic petri-dishes (100 mm). The inoculated petri-dishes were packed in a plastic bag and incubated at 26°C for two weeks. A small (about 2 mm in diameter) reddish-pink colony, spotted after two weeks was picked up and subcultured on slanted YMG agar (yeast extract 4 g, malt extract 10 g, glucose 4 g, Bacto agar 20 g and distilled water 1 liter, pH 6.8) and incubated at 26°C. After checking purity of the culture, it was processed for various biological activities and taxonomic characteristics.

TLC Bioassay for the Detection of the Biological Activity

The crude extract obtained from the mycelium was spotted on a TLC plate (5×20 cm, 0.2μm) and developed ($CHCl_3$-MeOH, 16:1, v/v). The plate was then marked under UV and visible light for individual spots or regions. The spores of the agar slant culture of the test organism was suspended in 10 ml of sterile physiological saline. Aliquots of 50 μl of this spore suspension were mixed with 10 ml of melted EMMON's medium held at 45°C and poured uniformly over the developed TLC plates and allowed to set. The plates were then placed in a moist chamber and incubated at 26°C for 24~72 hours and the zone of inhibition was recorded for the respective spots of the plate. The test organisms used were *C. albicans*, *A. fumigatus* and *Cladosporium* spp. and the growth medium was Potato Dextrose Agar (PDA, 39 g/liter).

Antifungal Activities

Initially, the cultures were grown in 500-ml baffled bottom Erlenmeyer flasks containing 100 ml liquid YMG medium (same as above but devoid of agar) and placed on a rotary shaker (200 rpm) at 26°C. After a week, the flasks were removed from the shaker and 0.03 ml of the culture broth was placed in the center of a petri-dish containing 20 ml EMMON's medium (Neopeptone 10 g, glucose 20 g, Bacto agar 20 g, distilled water 1 liter, pH 6.8) seeded with *ca.* 10^4 cfu of the test organism. An equal amount of the uninoculated culture medium was placed at a corner of the petri-dish as control. During the initial screening the test organisms included yeast phase of *Candida albicans* and conidia of *Aspergillus fumigatus*. The petri-dishes were incubated at 26°C for 48 hours, thereafter a clear zone of inhibition characterized by absence of the growth of the test organism around the broth droplet, was measured. During the follow-up studies, larger batches were grown in two-liter baffled bottom flasks containing 400 ml of the liquid YMG medium. After

one week, (pH *ca.* 7.0) the microbial growth was harvested by centrifugation or filtration and the cell mass homogenized in $CHCl_3$-MeOH (4:1, v/v).

Known amounts of the purified substance were dissolved in DMSO and serial dilutions prepared in the same solvent. A 20 μl volume of each dilution was placed on the surface of EMMON's agar in petri-dishes seeded with various test strains of pathogenic and saprophytic fungi. The antibacterial activity was monitored using Mueller-Hinton agar. The minimum inhibitory concentration (MIC) was determined by incorporating known amount of the DMSO dissolved purified substance into EMMON's liquid medium (same as stated earlier but devoid of agar) contained in test tubes. The tubes were inoculated with *ca.* 2×10^3 cfu of the test organisms. The inoculated tubes were incubated at 26°C and the results recorded after 2 to 5 days depending upon the growth pattern of the test organisms (certain fungi grow faster than others). Tubes inoculated in an identical manner but devoid of the active substance served as controls. MIC was the lowest concentration required to completely inhibit growth of the test organisms (Table 1).

Fermentation of the Strain 43097

It was grown in shake culture (130 rpm) at 26°C in a 2-liter baffled bottom Erlenmeyer flasks containing 400 ml of the liquid YMG medium. The medium after sterilization had a pH 6.5~7.0 and was incubated with 5~10 ml of a thick suspension of the homogenized colonies. Within the 2~5 day incubation period the medium became orange-red. The mycelium was harvested by cold centrifugation (10,000 rpm, 10 minutes) followed by vacuum filtration through a sintered glass filter (fine). The pH of the medium remained at neutral throughout the growth period. The red orange mycelium was vacuum dried to remove as much broth as possible followed by extraction of spartanamicins (Fig. 1).

Spartanamicin B

Red orange amorphous solid, $C_{42}H_{54}O_{16}N$ ($(M+H)^+$, calcd: 828.3453, found: 828.3448); mp 159~161°C; IR (KBr) cm^{-1} 3463, 2939, 1736, 1601, 1452, 1295, 1010; UV (MeOH-H_2O, pH 7.42) λ (nm) (ε) 523 (7,474), 491 (12,033), 481 (11,344), 289 (7,156), 257 (19,614), 234 (39,812); UV (MeOH-H_2O, pH 12.36) λ (nm) (ε) 592 (9,401), 516 (11,000), 496 (14,511), 488 (13,497), 486 (13,458), 297 (8,816), 290 (8,855), 258 (22,001), 240 (22,781); ^1H and ^{13}C NMR (Tables 2 and 3); MS (FAB+, % intensity): 828.3448 (55, $C_{42}H_{54}O_{16}N$, M+H), 586.2304 (50, $C_{30}H_{34}O_{12}$), 393.0980 (100, $C_{22}H_{17}O_7$), 349.1073 (30, $C_{21}H_{17}O_5$), 335.0914 (25, $C_{20}H_{15}O_5$); MS (FAB−; % intensity): 826 (45, M−H), 810 (5), 614 (5), 410 (100), 307 (60); MS (CI+, % intensity): 828 (10, $(M+H)^+$), 810 (5), 586 (5), 418 (10), 392 (100), 377 (15), 361 (20).

Spartanamicin A

Orange amorphous powder, $C_{42}H_{51}O_{16}N$; mp 174~176°C; IR (KBr) cm^{-1} 3510, 2980, 2950, 2830, 2780, 1740, 1645, 1602, 1450, 1400, 1320, 1215, 1005; UV (MeOH-H_2O, 95:5) λ (nm) (ε) 525 (9,334), 510 (10,628), 491 (13,757), 460 (11,111), 289 (9,090), 257 (24,632), 233 (46,830); ^1H and ^{13}C NMR (Tables 2 and 3); MS (FAB+, % intensity): 826.1420 (90, M+H), 393 (42), 309 (95).

Spartanone, the Aglycone from Spartanamicins A and B

Spartanamicin B (100 mg) was dissolved in HCl in MeOH (5 N, 10 ml) and kept at room temperature (1 hour). A TLC check of the reaction mixture showed the disappearance of the starting material. It was dried *in vacuo* and purified by preparative TLC (silica plate, 16:1 $CHCl_3$-MeOH). The resulting red orange product, spartanone (50 mg) had mp 248~249°C; UV (MeOH) λ (nm) (ε) 201 (15,756), 233 (38,894), 257 (19,287), 289 (6,928), 295 (6,797), 480 (11,155), 490 (11,823), 509 (8,988), 523 (7,329); IR (KBr) cm^{-1} 3450, 3130, 2930, 2975, 1770, 1640, 1600, 1455, 1410, 1300; ^1H NMR ($CDCl_3$): δ 12.89, 12.78 and 12.10 (1H each, all s, all exchanged with D_2O, phenol), 7.70 (1H, s, H-11), 7.30 (1H, d, J=9.4 Hz, H-3), 7.20 (1H, d, J=9.4 Hz, H-2), 5.38 (1H, d, J=4.7 Hz, H-7), 4.11 (1H, s, H-10), 3.72 (3H, s, $COOCH_3$), 2.56 (1H, dd, J=15.0, 5.2 Hz, H-8eq), 2.35 (1H, dd, J=14.90, 5.2 Hz, H-8ax), 1.74 (1H, m, J=7.3 Hz, 13a), 1.60 (1H, m, J=7.2 Hz, 13b), 1.27 (2H, br s, OH×2, exchanged with D_2O), 1.13 (3H, t, J=7.3 Hz, 14-CH_3); MS (CI+, % intensity): 393 (70, M^+H-2H_2O), 392 (100, M^+-2H_2O), 377 (15, $M^+-2H_2O-CH_3$), 361 (20, $M^+-2H_2O-OCH_3$), 333 (5). Similarly, spartanamicin A produced an aglycone identical to spartanaone, upon treatment with HCl in MeOH.

Acknowledgment

This research is a contribution from the Michigan State University Agricultural Experiment Station and was funded by a grant from XeChem, formerly a division of LyphoMed, Chicago and from the Research Excellence Fund from the State of Michigan.

References

1) LEE, M. D.; T. S. DUNNE, M. M. SIEGAL, C. C. CHANG, G. O. MORTON & D. B. BORDERS: Calichemicins, a novel family of antitumor antibiotics. 1. Chemistry and partial structure of calichemicin γ_1. J. Am. Chem. Soc. 109: 3464~3466, 1987
2) LEE, M. D.; T. S. DUNNE, C. C. CHANG, G. A. ELLESTAD, M. M. SIEGEL, G. O. MORTON, W. J. MCGAHREN & D. B. BORDERS: Calichemicins, a novel family of antitumor antibiotics. 2. Chemistry and structure of calichemicin γ_1. J. Am. Chem. Soc. 109: 3466~3468, 1987
3) GOLIK, J.; J. CLARDY, G. DUBAY, G. GROENEWOLD, H. KAWAGUCHI, M. KONISHI, B. KRISHNAN, H. OHKUMA, K. SAITOH & T. W. DOYLE: Esperamicins, a novel class of potent antitumor antibiotics. 2. Structure of esperamicin X. J. Am. Chem. Soc. 109: 3461~3462, 1987
4) WAGMAN, G. H.; G. M. LUEDEMANN & M. J. WEINSTEIN: Fermentation and isolation of everninomicin. Antimicrob. Agents Chemother.-1964: 33~37, 1965
5) GANGULY, A. K. & A. K. SAKSENA: Hydrolysis products of everninomicin B. J. Chem. Soc. Chem. Commun. 1973: 531~532, 1973
6) BICKEL, H.; G. E. HALL, W. KELLER-SCHIERLEIN, V. PRELOG, E. VISCHER & A. WETTSTEIN: 261 Stoffwechselprodukte von Actinomyceten Uber die Konstitution von Ferrioxamin B. Helv. Chim. Acta 43: 2129~2138, 1960
7) PRELOG, V. & A. WALSER: Stoffwechselprodukte von Actinomyceten—Uber die Synthese der Ferrioxamine B und D. Helv. Chim. Acta 45: 631~637, 1962
8) OKI, T.; A. MATSUZAWA, A. YOSHIMOTO, K. NUMATA, I. KITAMURA, S. HORI, A. TAKAMATSU, H. UMEZAWA, M. ISHIZUKA, H. NAGANAWA, H. SUDA, M. HAMADA & T. TAKEUCHI: New antitumor antibiotics, aclacinomycins A and B. J. Antibiotics 28: 830~834, 1975
9) OKI, T.; N. SHIBAMOTO, Y. MATSUZAWA, T. OGASAWARA, A. YOSHIMOTO, I. KITAMURA, T. INUI, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: Production of nineteen anthracyclic compounds by *Streptomyces galilaeus* MA144-M1. J. Antibiotics 30: 683~687, 1977
10) OKI, T.; I. KITAMURA, A. YOSHIMOTO, Y. MATSUZAWA, N. SHIBAMOTO, T. OGASAWARA, T. INUI, A. TAKAMATSU, T. TAKEUCHI, T. MASUDA, M. HAMADA, H. SUDA, M. ISHIZUKA, T. SAWA & H. UMEZAWA: Antitumor anthracycline antibiotics, aclacinomycin A and analogues. I. Taxonomy, production, isolation and physicochemical properties. J. Antibiotics 32: 791~800, 1979
11) OKI, T.; I. KITAMURA, Y. MATSUZAWA, N. SHIBAMOTO, T. OGASAWARA, A. YOSHIMOTO, T. INUI, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: Antitumor anthracycline antibiotics, aclaninomycin A and analogues. II. Structural determination. J. Antibiotics 32: 801~819, 1979
12) SOGA, K.; H. FURUSHO, S. MORI & T. OKI: New antitumor antibiotics: 13-Methylaclacinomycin A and its derivatives. J. Antibiotics 34: 770~773, 1981
13) TANAKA, H.; T. YOSHIOKA, Y. SHIMAUCHI, Y. MATSUSHITA, Y. MATSUZAWA, T. OKI & T. INUI: Preparation of 4- and 6-*O*-methylaclacinomycin derivatives and their antitumor activities. J. Antibiotics 34: 905~908, 1981
14) MATSUZAWA, Y.; T. OKI, T. TAKEUCHI & H. UMEZAWA: Structure-activity relationships of anthracyclines relative to cytotoxicity and effects on macromolecular synthesis in L1210 leukemia cells. J. Antibiotics 34: 1596~1607, 1981
15) BIEBER, L. W.; Á. A. DA SILVA FILHO, J. F. DE MÉLLO, O. G. DE LIMA, M. S. DO NASCIMENTO, H. J. VEITH & W. VON DER SAAL: Desaminoanthracyclines from the antibiotic complex ciclamycin. J. Antibiotics 40: 1335~1338, 1987