JOC
The Journal of Organic Chemistry

VOLUME 65, NUMBER 10 MAY 19, 2000

© Copyright 2000 by the American Chemical Society

Articles

Hybrid Anthracyclines from a Genetically Engineered *Streptomyces galilaeus* Mutant

Tero J. Kunnari,[†] Kristiina P. J. Ylihonko,[†,‡] Karel D. Klika,[§] Pekka I. Mäntsälä,[‡] and Juha M. L. Hakala*[†,||]

Galilaeus Oy, P.O. Box 113, 20781 Kaarina, Finland, Department of Biochemistry, University of Turku, 20014 Turku, Finland, and Department of Chemistry, University of Turku, 20014 Turku, Finland

Received February 24, 1998 (Revised Manuscript Received February 2, 2000)

The genetic engineering of antibiotic-producing *Streptomyces* strains is an approach that is emerging and ready to become established as a successful methodology in developing analogues of the original, pharmaceutically important, natural products obtained from the organisms. The current report highlights this succes by demonstrating the high-level production of novel anthracyclines. The biosynthetic pathways of the nogalamycin-producing *Streptomyces nogalater* and the aclacinomycin-producing *S. galilaeus* were combined by transferring the genes of *S. nogalater* polyketide synthetase into a nonproducing *S. galilaeus* mutant. The resulting anthracycline antibiotics that were produced possessed structural features characteristic of compounds from both of the undoctored *Streptomyces* strains.

Introduction

Anthracycline antibiotics play an important role in cancer chemotherapy, and because of their usefulness, and with the desire for a broader therapeutic index, these compounds have stimulated an ongoing search for compounds with improved properties.[1] Traditionally, one approach has been the serendipidous screening of bacteria isolated from soil. This has resulted in the clinical application of several anthracyclines, of which daunorubicin[2] and aclacinomycin[3] A are examples. The mutated anthracycline-producing strains can also result in novel compounds, some of which have found their way into clinical use, including doxorubicin, obtained from a mutant of daunorubicin-producing *S. peucetius*.[4] The synthetic modification of a microbially produced anthracycline substrate has also resulted in successful developments, of which menogaril[5] derived from nogalamycin **1** and idarubicin[6] derived from daunorubicin are examples. However, an emerging technology for novel anthracycline production is the combination of the biosynthetic pathways from different *Streptomyces* strains by transfer of those genes responsible for metabolite synthesis from one organism into another, producing or blocked, host strain. Secondary metabolite products of the hybrid strain carry structural elements derived from both the plasmid and

† Galilaeus Oy.
‡ Department of Biochemistry, University of Turku.
§ Department of Chemistry, University of Turku.
|| E-mail: juha.hakala@galilaeus.fi.

(1) For reviews of anthracyclines, see: Weiss, R. B. *Semin. Oncol.* **1992**, *19*, 670.
(2) DiMarco, A.; Silverstrini, R.; Gaetani, M.; Soldati, M.; Orezzi, P.; Dasdia, T.; Scarpinato, B. M.; Valentini, L. *Nature* **1964**, *201*, 706.
(3) Oki, T.; Matsuzawa, Y.; Yoshimoto, A.; Numata, K.; Kitamura, I.; Hori, S.; Takamatsu, A.; Umezawa, H.; Ishizuka, M.; Naganawa, H.; Suda, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1975**, *28*, 830.
(4) Arcamone, F.; Franceschi, G.; Penco, S. *Tetrahedron. Lett.* **1969**, *13*, 1007.
(5) Arcamone, F.; Cassinelli, G. *Curr. Med. Chem.* **1998**, *5*, 391.
(6) Cabri, W.; Bernardinis, S. D.; Francalanci, F.; Penco, S. *J. Chem. Soc., Perkin Trans. 1* **1990**, 428.

10.1021/jo980345o CCC: $19.00 © 2000 American Chemical Society
Published on Web 04/14/2000

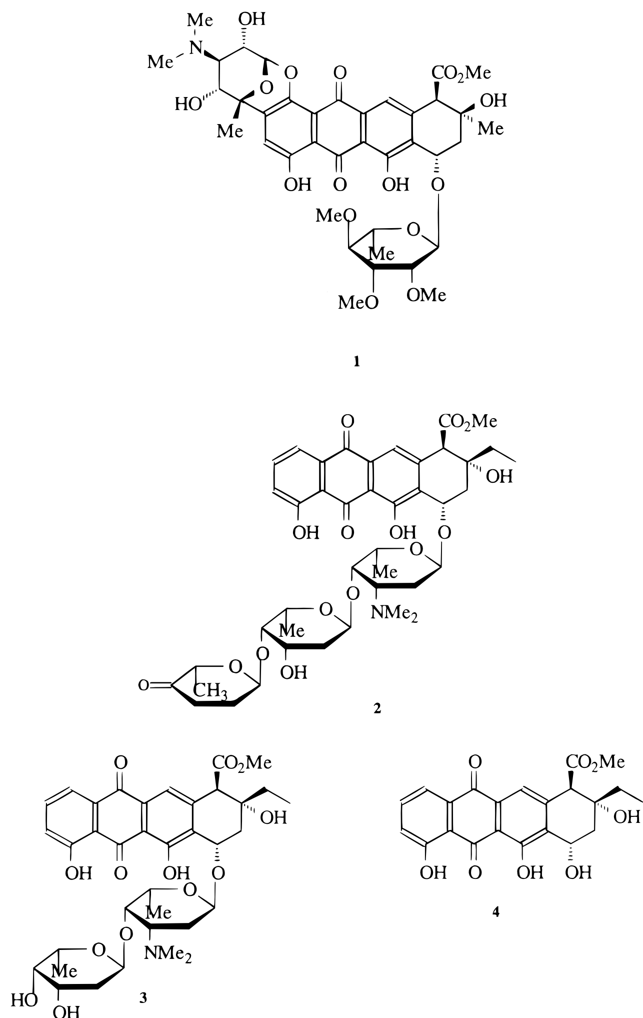

Figure 1. Structures of nogalamycin **1**, aclacinomycin A **2**, aclacinomycin S **3**, and aklavinone **4**.

the host. The first demonstration of this technology in the field of anthracyclines was the transfer of genes from *S. purpurascens* responsible for the last steps in biosynthesis of rhodomycin biosynthesis into *S. galilaeus* (an aclacinomycin-producing strain), resulting in aclacinomycin-type anthracyclines possessing a modified rhodomycine-type aglycon and glycosylation profile determined by the host, *S. galilaeus*.[7] Another recent demonstration was the introduction of *S. nogalater* genes into *S. lividans* TK24, also resulting in hybrid products with structural features that could be associated with either parent.[8]

S. nogalater produces nogalamycin **1**,[9] an anthracycline differing in several structural aspects from aclacinomycins produced by *S. galilaeus* (Figure 1). The major difference in the biosynthesis of aclacinomycins and nogalamycin is that the polyketide starter unit is acetate in the nogalamycin pathway[10] as opposed to propionate in the aclacinomycin pathway. As a consequence of this difference, the side chain at C-9 is ethyl in the aclacinomycins and methyl in nogalamycin **1**. The absolute stereochemistry at C-9 in aclacinomycins and nogalamycin **1** also differs, whereas the other two stereocenters of the aglycon, C-7 and C-10, have the same absolute stereochemistry. The pattern of glycosylation also differs markedly in the products of *S. galilaeus* and *S. nogalater*. Nogalamycin **1** has two separate sugars attached to the anthraquinone substrate: a conventional attachment at the C-7 oxygen and a second sugar forming a fused bicycle by attachement to the aromatic part of the aglycon via an ether bridge and a carbon−carbon bond.[10] In aclacinomycins, only one sugar moiety consisting of one to three residues is attached directly to the anthraquinone substrate via a C-7 oxygen link. In this paper we report the production of anthracyclines from *S. galilaeus* nonproducing mutant H028[11] complemented with the plasmid pSY15 containing the polyketide synthase (PKS) genes from *S. nogalater*.[12] This combination of the biosynthetic pathways of the two strains results in the production of hybrid antibiotics.

Notable in this work is that it does not only further demonstrate the rapidly emerging technology for the production of hybrid compounds but in particular it exemplifies the compatibility of the stereospecificities of the two biosynthetic pathways.

Results and Discussion

Isolation and NMR Spectroscopic Characterization of the Compounds. Extraction of the fermentation broth of H028/pSY15 resulted in a mixture of compounds **5**−**12**, with similar UV spectra and which were typical for compounds containing anthraquinone chromphores. On the basis of their chromatographic behavior during isolation, compounds **5**−**12** were initially classified as aglycons, amino glycosides, or neutral glycosides. 1D ^{1}H and ^{13}C NMR spectral assignments (see Tables 1−3) were made using a conventional combination of DEPT, TOCSY, HSQC, HMBC, and NOE difference measurements.

Isolate **5**, a yellow solid of molecular weight 398, was readily identified as auramycinone on the basis of both ^{1}H and ^{13}C spectra which were also consistent with the earlier published data.[11] Resonances arising from the aliphatic ring A were investigated to reveal a difference in relative stereochemistry between nogalamycinone and auramycinone. It showed one ABMX system (H-8), two methyl singlets (H-15 and H-13), and one long-range-coupled methine signal (H-10) in the proton spectrum. Three hydrogen-bonded hydroxyl proton signals were observed in the spectrum. Irradiation at the resonance of the hydroxyl proton at C-9 and recording the saturation difference spectrum revealed that the hydroxyl group at C-7 resonated at 3.6 ppm.

The ^{1}H NMR spectra of the two glycosidic isolates, **6** and **7**, suggested that the aglycon part was auramycinone **5**; this was confirmed chemically by hydrolysis of both **6** and **7** to yield **5**. Compounds **6** and **7** each showed two anomeric protons resonating between 5.5 and 5.0 ppm corresponding to disaccharide units. The sugar order and attachement at C-7 were established by HMBC and NOE experiments. Important inter substructural correlations were observed between pairs H-7/H-1′ and H-4′/H1″,

(7) Niemi, J.; Ylihonko, K.; Hakala, J.; Pärssinen, R.; Kopio, A.; Mäntsälä, P. *Microbiology* **1994**, *140*, 1351.
(8) Kunnari, T.; Kantola, J.; Ylihonko, K.; Klika, K. D.; Mäntsälä, P.; Hakala, J. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1649.
(9) Wiley, P. F.; Kelly, R. B.; Caron, E. L.; Wiley, V. H.; Johnson, J. H.; MacKellar, F. A.; Mizsak, S. A. *J. Am. Chem. Soc.* **1977**, *99*, 542.
(10) Wiley, P. F.; Elrod, D. W.; Marshall, V. P. *J. Org. Chem.* **1978**, *43*, 3457.
(11) Ylihonko, K.; Hakala, J.; Niemi, J.; Lundell, J.; Mäntsälä, P. *Microbiology* **1994**, *140*, 1359.
(12) Ylihonko, K.; Tuikkanen, J.; Jussila, S.; Cong, L.; Mäntsälä, P. *Mol. Gen. Genet.* **1996**, *251*, 113.

Table 1. ¹H NMR Spectral Data[a] for Compounds 4, 5, 9, and 10

site	**4**	**5**	**9**	**10**
1	7.80 dd, 7.4, 1.0	7.84 dd, 7.5, 1.2	7.85 dd, 7.5, 1.2	7.85 dd, 7.6, 1.2
2	7.66 t, 8.3, 7.4	7.71 dd, 8.5, 7.5	7.73 dd, 8.5, 7.5	7.73 t, 8.5, 7.6
3	7.37 dd, 8.3, 1.0	7.32 dd, 8.5, 1.2	7.35 dd, 8.5, 1.2	7.34 dd, 8.5, 1.2
4-OH	11.92 s	11.97 s	11.91 s	11.95 s
6-OH	12.69 s	12.74 s	12.59 s	12.54 s
7	5.34 ddd, 5.4, 3.6,1.7	5.39 brs		
7-OH	3.31 d, 3.6	3.64 brs		3.66 brs
8A	2.51 dd, 15.1, 5.4	2.63 dd, 15.0, 5.1	3.27 dd, 17.8, 2.6	2.69 s
8B	2.23 ddd, 15.1, 1.7, 1.5	2.24 ddd, 15.0, 2.1, 1.0	3.12 dd, 17.8, 9.1	
9			4.42 m	
9-OH	3.89 s	4.06 s	3.06 brs	
10	4.05 d, 1.5	4.06 d, 1.0	3.88 s, 3H	3.88 s, 3H
11	7.68 s	7.70 s	7.72 s	7.71 s
13		1.42 s, 3H	1.29 d, 6.5, 3H	
13A	1.68 dq, 14.5, 7.5			
13B	1.54 dq, 14.5, 7.5			
14	1.06 t, 7.5, 3H			
15		3.72 s, 3H	3.75 s, 3H	3.72 s, 3H
16	3.67 s, 3H			

[a] Chemical shift (ppm), multiplicity, J_{HH} (Hz), integration (only indicated if other than 1H).

Table 2. ¹H NMR Spectral Data[a] for Compounds 1, 3, 6, and 7

site	**1**	**3**	**6**	**7**
1		7.84 d, 7.7	7.86 dd, 7.6, 1.2	7.84 dd, 7.6, 1.4
2		7.68 t, 8.4, 7.7	7.70 t, 8.4, 7.6	7.70 t, 8.3, 7.6
3	7.10 s	7.31 d, 8.3, 1.2	7.32 dd, 8.4, 1.2	7.32 dd, 8.3, 1.4
4-OH	11.69 brs	12.05 s	12.03 s	12.03 s
6-OH	12.78 brs	12.69 brs	12.73 s	12.70 brs
7	5.17 dd, 6.4, 4.3	5.27 brs	5.26 brs	5.28 brs
8A	2.69 dd, 14.1, 6.4	2.52 dd, 14.9, 4.2	2.62 dd, 14.5, 4.2	2.61 dd, 14.9, 4.1
8B	2.09 dd, 14.1, 4.3	2.29 d, 14.9	2.20 d, 14.5	2.20, d, 14.9
9-OH	3.32 brs	4.56 brs	4.38 s	4.78 brs
10	3.98 s	4.12 s	4.11 s	4.11 s
11	6.98 s	7.70 s	7.68 s	7.67 s
13A		1.75 m		
13B		1.51 m		
13	1.59 s		1.39 s, 3H	1.39 s, 3H
14	-	1.09 t, 7.4, 3H		
15	3.75 s, 3H		3.72 s, 3H	3.71 s, 3H
16		3.70 s, 3H		
1′	5.77 d, 3.7	5.52 brs	5.50 d, 3.4	5.54 d, 3.0
2′	4.24 dd, 10.7, 3.7	1.87 dd, 6.8, 2.2, 2H	1.95 dt, 9.0, 3.9, 2H	1.85 m, 2H
3′	3.10 brs	3.64 brs	1.77 dt, 9.0, 3.0, 2H	3.63 brs
3′-NMe₂	2.63 brs, 6H	2.18 brs, 6H		2.18 brs, 6H
4′	3.63 d, 10.5	3.74 brs	3.75 brs	3.74 brs
5′		4.00 q, 6.8	4.17 q	4.01 q, 7.1
6′	1.68 s, 3H	1.28 d, 6.8, 3H	1.27 d, 3H	1.21 d, 7.1, 3H
1″	5.49 s	5.01 brs	4.97 d, 3.3	5.01 d, 2.8
2″	3.55 brs	2.08 m	2.07 dd, 12.0, 4.9, 2H	2.08 dd, 11.7, 4.6, 2H
2″-OMe	3.66 s, 3H			
3″		4.13 m	4.17 m	4.13 m
3″-OMe	3.28 s, 3H			
3″ Me	1.37 s, 3H			
4″	3.20 d, 9.8	3.75 brs	3.59 brs	3.62 brs,
4″-OMe	3.58 s, 3H			
5″	3.78 dq, 9.8, 6.1	4.54 q, 6.1	4.17 q	4.53 q, 7.1
6″	1.39 d, 6.1, 3H	1.20 d, 6.1, 3H	1.27 d, 3H	1.29 d, 7.1, 3H

[a] Chemical shift (ppm), multiplicity, J_{HH} (Hz), integration (only indicated if other than 1H).

revealing the arrangement and positions of sugar residues. The observed HMBC correlations for **4** is depicted in Figure 2. The small size of the couplings (3 Hz) for anomeric protons in both sugar residues suggests that glycosidig lingages are axial. The other obseved couplings of the sugar rings were typical for a chair conformation.[13] Sugar ring substitution of **6** and **7** is similar except that compound **6** has a dimethylamino group at position 3′ instead of a hydroxyl. Partial hydrolysis (0.5 M HCl in MeOH, rt, 1 h) of compound **6** produced a monosaccharide, with a dimethylamino group at position 3′. Both **6** and **7** were found to be novel structures.

Compound **8** is a biosynthetic precursor, leading after cyclization and reduction to **5**, and has been isolated recently from *S. argilaceus* A33 carrying pSY15.[8] Shunt product **9** described earlier from *S. lividans* TK24/pSY15 showed similarities to **8**, except with a second ketone group of the side chain reduced to a hydroxyl leading to a shunt product unable to cyclize to **5**.[14] Structure **10** has been described earlier and has one less extender unit

(13) Parkinson, J. A.; Sadler, I. H.; Pickup, M. P.; Tabor, A. B. *Tetrahedron* **1995**, *51*, 7215.

(14) Ylihonko, K.; Hakala, J.; Kunnari, T.; Mäntsälä, P. *Microbiology* **1996**, *142*, 1965.

Scheme 1. Products Obtained from H028/PSY15 Arranged According to the Proposed Biosynthetic Route

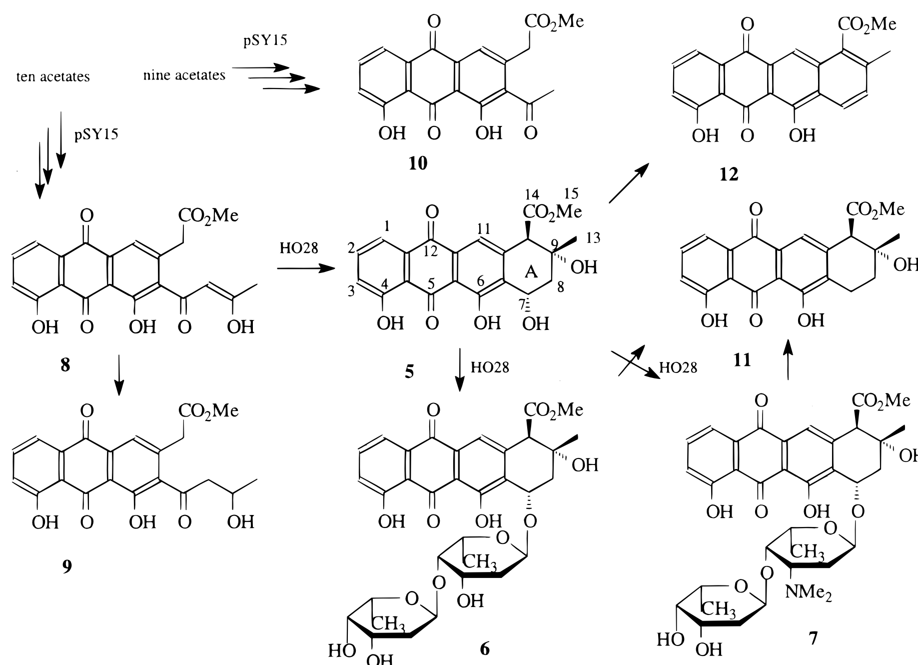

Table 3. ^{13}C NMR Spectral Data[a] for Compounds 1, 4, 5, 6, and 7

site	**1**	**4**	**5**	**6**	**7**
1	147.9(s)	120.2(d)	120.2(d)	120.9(d)	120.4(d)
2	137.8(s)	137.4(d)	137.4(d)	137.4(d)	137.7(d)
3	125.8(d)	124.8(d)	124.8(d)	124.8(d)	125.1(d)
4	155.7(s)	162.1(s)	162.4(s)	162.5(s)	162.7(s)
4a	116.0(s)	115.8(s)	115.5(s)	115.7(s)	115.6(s)
5	191.0(s)	192.7(s)	192.3(s)	192.6(s)	192.6(s)
5a	113.9(s)	114.7(s)	114.4(s)	114.6(s)	115.4(s)
6	162.0(s)	162.5(s)	161.1(s)	162.0(s)	160.1(s)
6a	130.9(s)	133.5(s)	132.4(s)	130.8(s)	135.2(s)
7	(d)	71.2(d)	62.4(d)	70.7(d)	71.6(d)
8	40.9(t)	32.5(t)	37.0(t)	36.6(t)	36.8(t)
9	(s)	66.6(s)	69.9(s)	69.7(s)	70.0(s)
10	56.8(d)	57.1(d)	57.8(d)	57.9(d)	57.8(d)
10a	143.6(s)	142.6(s)	142.3(s)	142.4(s)	141.9(s)
11	119.0(d)	120.9(d)	121.1(d)	120.2(d)	120.4(d)
11a	133.4(s)	132.9(s)	132.6(s)	132.9(s)	133.2(s)
12	179.5(s)	181.3(s)	180.9(s)	181.2(s)	180.8(s)
12a	114.0(s)	133.5(s)	133.2(s)	133.4(s)	133.7(s)
13	29.4(q)	30.2(t)	27.5(q)	27.2(q)	22.8(q)
14	172.0(s)	6.6(q)	171.3(s)	171.4(s)	170.5(s)
15	59.2(q)	171.3(s)	52.5(q)	52.5(q)	52.6(q)
16		52.5(q)			
1′	100.6(d)			101.8(d)	101.2(d)
2′	81.2(d)			36.6(t)	35.2(t)
3′	65.8(d)			65.4(d)	60.1(d)
3′NMe2	41.4(q)				43.2(q)
4′	71.0(d)			82.9(d)	81.4(d)
5′	75.5(s)			67.4(d)	68.2(d)
6′	24.0(q)			17.0(q)	16.8(q)
1″	97.2(d)			100.8(d)	100.2(d)
2″OMe	61.0(q)				
2″	81.6(d)			34.0(t)	33.7(t)
3″	78.2(s)			65.4(d)	66.5(d)
3″Me	15.0(q)				
3″OMe	59.2(q)				
4″	84.3(d)			70.9(d)	70.7(d)
4″OMe	51.5(q)				
5″	69.9(d)			67.3(d)	68.1(d)
6″	18.2(q)			16.7(q)	16.6(q)

[a] Chemical shift (ppm) and multiplicity.

than **8**.[8] Compounds **11** and **12** are typical degradation products of anthracyclines formed during the fermentation and workup. Reductive deglycosylation of **6** and **7** results in 7-deoxyauramycinone **11**. Elimination of two waters from **5** results in the fully aromatized bisanhydroauramycinone **12** (Scheme 1).

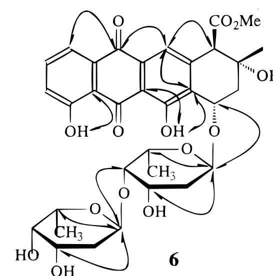

Figure 2. The most important observed HMBC correlations of **6**.

The relative stereochemistry of the ring A of aclacinomycins is (7*S*,9*R*,10*R*), and the ring has two possible minimum energy conformations, namely α- and β-half-chair.[15] However, the α-form is stabilized over the β-form by intramolecular hydrogen bonding from the C-9 hydroxyl to the etheral oxygen at C-7 and a lower electronic repulsion between the etheral oxygen at C-7 and the hydroxyl located on C-6. Internal hydrogen bonding for the C-9 hydroxyl to the C-7 hydroxyl oxygen in auramycinone **5** was indicated by the ^{1}H resonance of the C-9 hydroxyl not showing any chemical shift variation upon dilution of the NMR sample (0.02−0.22 M)—in agreement with the earlier X-ray diffraction studies on similar compounds.[15] The stereochemistry at C-9 in nogalamycin **1**, however, is of opposite configuration (i.e., 9*S*). To compare the stereochemistry of ring A between aklavinone **4**, auramycinone **5**, and nogalamycin **1**, their ^{1}H and ^{13}C chemical shifts and NOE spectra were measured.

The signal of the hydroxyl proton at C-9 in nogalamycin **1** was shifted to a higher field by 1 ppm compared to that of aklavinone **4** and auramycinone **5**, indicating the

(15) Penco, S.; Vigevani, A.; Tosi, C.; Fusco, R.; Borghi, D.; Arcamone, F. *Anti-Cancer Drug Des.* **1986**, *1*, 161.

lack of an intramolecular hydrogen bond which was present between the hydroxyl at C-9 and the oxygen at C-7. Major changes in chemical shifts in nogalamycin **1** as compared to auramycinone **5** were observed for the equatorial proton at C-8 (0.15 ppm upfield) and methyl at C-13 (0.12 ppm downfield). The couplings between the protons at C-8 and C-7 implied small changes in the conformation of ring A, propably due to the absence of hydrogen bonding in nogalamycin **1**. The ^{13}C NMR spectrum showed alterations of chemical shifts at C-8 (4 ppm upfield) and C-13 (2 ppm downfield). An NOE enhancement of about equal size was observed between protons at C-10/C-11 (14%) and C-10/C-13 (2.4%) in aklavinone **4** and auramycinone **5**, indicating the same relative stereochemistry. The NOEs for the same protons of nogalamycin **1** were 8% and 6%, respectively. The NOEs between the protons at C-7 and C-8 were of nearly equal magnitude for all three compounds. For a reference, the ^{1}H spectrum of aclacinomycin S **3** from the *S. galilaeus* mutant HO26 with sugar residues identical to those of **7** but with an aklavinone aglycon was measured (Table 2).

Biosynthetic Pathways. The proposed biosynthetic pathway of products from H028/pSY15 is shown in Scheme 1. The hybrid compounds produced by H028/pSY15 all have an acetate as the starter unit as determined by the PKS originating from *S. nogalater*. This results in the main product possessing a methyl as the C-9 side chain, as in the nogalamycin aglycon. Both aglycons of nagalamycin **1** and aclacinomycin consist of the starter unit and nine extender units which are acetates in all aromatic polyketide antibiotics. The *S. galilaeus* mutant choosing an acetate instead of the propionate as starter unit produce both auramycines consisting of 10 acetates and sulfurmycines consisting of 11 acetates.[16] Furthermore, a compound similar to the minor shunt product **10** with a ketopropyl side chain instead of the ketoethyl has been isolated from the fermentation broth of a *S. galilaeus* mutant.[17] The same is true for the *S. peucetius* mutants choosing an acetate as a starter unit instead of a propionate, thus producing feudomycines: feudomycine A consist of 11 acetates whereas feudomycine D consists of 10 acetates.[18] The lack of fidelity is propably due to a flexible PKS. In the case of H028/pSY15, anthracyclines composed of 10 acetates were mainly found, suggesting that *S. nogalater* confers higher fidelity to the polyketide synthesis, though a minor amount of **8** was obtained.

The stereochemistry at C-9 of the metabolites **5**, **6**, and **7** was shown to be similar to that found in aklavinone **4** but different from the C-9 stereochemistry of nogalamycin **1** because the gene responsible for the closure of the ring A of the aglycon, and hence the concomitant determination of the stereochemistry, is not present in pSY15, leaving the stereochemistry to be determined by the host strain HO28. Additionally, the two glycosides **6** and **7** produced by HO28/pSY15 resemble aclacinomycins as they have only one sugar moiety attached at C-7. In both glycosides, the sugars are those represented in the aclacinomycins, implying that the enzymes responsible for the biosynthesis of the sugar moieties all originate from the parent strain *S. galilaeus* HO28.

Experimental Section

General Procedures and Materials. NMR spectra were recorded on a 400 MHz spectrometer. ^{1}H and ^{13}C NMR samples were internally referenced to TMS. All NMR studies were carried out at 25 °C in $CDCl_3$ under neutral pH = 7.0 conditions. MS spectra were taken in the EI mode. UV spectra were recorded in methanol. Analytical TLC was done with precoated Kieselgel glass plates. HPLC separations and quantitations were carried out using a LiCHroCART RP-18 (Lichrosphere, 5 μm, 4.6 × 250 mm) column. Cultures of strain H028/pSY15 were maintained on ISP-4 agar dishes supplemented with thiostrepton (50 μg/mL).

Fermentation. The fermentation was carried out for 5 days in a jar fermentor in 10 L of E1 medium consisting of glucose, starch, Pharmamedia, yeast extract, calcium carbonate, sodium chloride, $MgSO_4$, and K_2HPO_4 in tap water. The aeration was at 10 L min $^{-1}$ with stirring at 500 rpm.

Isolation of Compounds. Prior to the separation, the pH of the culture was adjusted to 7.0 with 1 M phosphate buffer. The fermentation culture was centrifuged to separate mycelia from the broth. The mycelia was extracted twice with methanol (500 mL). Methanol extracts and broth were combined and extracted twice with diclormethane (1500 mL). Combined dichloromethane extracts were vacuum-concentrated to give a viscous residue, which was dissolved in ethyl acetate and precipitated with hexane, to give 2.0 g of yellow powder.

The precipitate was loaded onto a silica flash column and developed with 0.5% acetic acid in chloroform with increasing amounts of methanol (0−15)%. Each fraction was further purified on a silica gel column, using the solvent system $CHCl_3$−AcH (99.7:0.3) for the aglycons and $CHCl_3$−MeOH−AcH−H_2O (83:10:5:1.6) for the glycosides. The amounts of isolated pure compounds were 500 mg (**6**), 450 mg (**5**), 150 mg (**7**), 30 mg (**11**), 10 mg (**9**), and 5 mg (**10**). Mass spectral data, EI, *m/z* (relative intensity) for compounds **5**, 398 (M^+, 13), 380 (22), 362 (100), 347 (17), 331 (32), 321 (47); **10**, 354 (M^+, 100), 323 (15), 307 (57), 294 (88), 251 (8); **9**, 398 (M^+, 30), 353 (45), 323 (100), 307 (55), 295 (35), 280 (23); **8**, 396 (M^+, 25), 378 (23), 364 (20), 319 (100), 307 (75), 279 (45).

JO980345O

(16) Fujiwara, A.; Hoshino, T.; Tazoe, M.; Fujiwara, M. *J. Antibiot.* **1981**, *34*, 608.
(17) Oki, T.; Matsuzawa, Y.; Kiyoshima, K.; Yoshimoto, A.; Naganawa, H.; Takeuchi,., Umezawa, H. *J. Antibiot.* **1981**, *34*, 783.
(18) Tobe, H.; Yoshimoto, A.; Ishikura, T.; Naganawa, H.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1982**, *35*, 1641.