JOURNAL OF BACTERIOLOGY, June 1996, p. 3384–3388 Vol. 178, No. 11
0021-9193/96/$04.00+0

Cloning, Sequencing, and Analysis of Aklaviketone Reductase from *Streptomyces* sp. Strain C5

MICHAEL L. DICKENS, JINGSONG YE,† AND WILLIAM R. STROHL*

Department of Microbiology, The Ohio State University, Columbus, Ohio 43210

Received 22 January 1996/Accepted 15 March 1996

DNA sequence analysis of a region of the *Streptomyces* sp. strain C5 daunomycin biosynthesis gene cluster, located just upstream of the daunomycin polyketide biosynthesis genes, revealed the presence of six complete genes. The two genes reading right to left include genes encoding the potentially translationally coupled gene products, an acyl carrier protein and a ketoreductase, and the four genes reading divergently, left to right, include two open reading frames of unknown function followed by a gene encoding an apparent glycosyltransferase and *dauE*, encoding aklaviketone reductase. Extracts of *Streptomyces lividans* TK24 containing recombinant DauE catalyzed the NADPH-specific conversion of aklaviketone, maggiemycin, and 7-oxodaunomycinone to aklavinone, ε-rhodomycinone, and daunomycinone, respectively. Neither the product of *dauB* nor that of the ketoreductase gene directly downstream of the acyl carrier protein gene demonstrated aklaviketone reductase activity.

Daunomycin (daunorubicin), adriamycin (doxorubicin), carminomycin, and aclacinomycin A (aclarubicin) are clinically important anthracycline chemotherapeutic agents of the polyketide class of antibiotics. These compounds are synthesized by condensation of nine extender units derived from malonyl coenzyme A onto a propionyl moiety to make a theoretical C-21 polyketide intermediate (27, 28), which is reduced at C-9 (28), cyclized, and aromatized to form aklanonic acid, the first known stable intermediate in these pathways (13, 27, 28). The free carboxyl group of aklanonic acid is then methylated by the *dauC* gene product, and the resulting methylester is cyclized by the *dauD* gene product to form the 7-oxo compound aklaviketone (3, 6, 10), which is then reduced by the *dauE* gene product (3, 6) to form aklavinone (Fig. 1), the last intermediate common to both aclacinomycin and daunomycin biosynthesis (27). Mutants of *Streptomyces* sp. strain C5 in which aklaviketone reductase activity is blocked (*dauE* mutants) accumulate the purple-pigmented compound maggiemycin (25), which is produced as a shunt product of aklaviketone hydroxylated at C-11 (3, 6, 10).

Aclacinomycin A is formed from aklavinone via multiple glycosylation steps while the structurally related anthracyclines carminomycin, daunomycin, and doxorubicin are formed by hydroxylation at C-11 prior to glycosylation and additional modifications to form the final compounds (27). Here we describe the isolation, sequence analysis, and characterization of *dauE* from *Streptomyces* sp. strain C5 encoding aklaviketone reductase, the final enzymatic step common to both aclacinomycin and daunomycin (or doxorubicin) biosynthesis, as well as the structure and potential function of the genes clustered with *dauE*.

Bacterial strains and growth conditions. *Streptomyces* sp. *dauE* mutant strains SC5-24, SC5-110, and SC5-111 have been described previously (2, 3). *Streptomyces lividans* TK24 (17) was obtained from D. A. Hopwood. *Streptomyces lividans* TK24 was grown in YEME medium supplemented with 20% sucrose (17). *Streptomyces* sp. strain C5 *dauE* mutants were grown in nitrate-defined medium plus yeast extract (NDYE) as described previously (3, 6). Strains carrying streptomycete plasmid pANT849 (7), pWHM3 (30), or a derivative were grown and stored on plates containing 40 μg of thiostrepton per ml. When liquid media were used to grow recombinant streptomycetes, 10 μg of thiostrepton per ml was added.

Genetic procedures. *Escherichia coli* JM83 was used to propagate plasmids for sequencing and restriction analyses by standard methods (22). All procedures for genetic manipulation of the streptomycetes, including DNA sequencing, have been described previously (17, 32). DNA and deduced gene products were analyzed by the algorithms of the Wisconsin Genetics Computer Group package (8). Deduced gene products also were compared with those in the databases by using both Genetics Computer Group programs (8) and the BLAST algorithm (1). Plasmids used and constructed in this work are listed in Table 1.

Open reading frames in sequenced DNA. We recently reported the isolation of ca. 30 kbp of DNA from the daunomycin-producing strain, *Streptomyces* sp. C5, within which a cluster of genes encoding daunomycin biosynthesis polyketide synthase were located (32). Subsequently, we identified the *dauC* and *dauD* genes, encoding aklanonic acid methyltransferase and aklanonic acid methyl ester reductase, respectively, the enzymes required for generation of aklaviketone, clustered with the daunomycin polyketide synthase genes (10). This same gene arrangement has been found in the daunomycin- and doxorubicin-producing species *Streptomyces peucetius* (15, 21).

Approximately 6.0 kbp of DNA directly adjacent to the *Streptomyces* sp. strain C5 polyketide synthase biosynthesis gene cluster was sequenced (Fig. 2). Within this DNA, six complete open reading frames that contained codon usage and G+C content in the third position within codons typical for streptomycetes were found (4, 31). Two of these genes, one each encoding an apparent ketoreductase (*orf1*) and an acyl

* Corresponding author. Mailing address: Department of Microbiology, Ohio State University, 484 West 12th Ave., Columbus, OH 43210-1292. Phone: (614) 292-1919. Fax: (614) 292-0316. Electronic mail address: wstrohl@magnus.acs.ohio-state.edu.

† Current address: Division of Hematology, St. Louis University Medical Center, St. Louis, Mo.

Substrates:	R_1	R_2	R_3
Aklaviketone	–H	$–COOCH_3$	–H
Maggiemycin	–OH	$–COOCH_3$	–H
7-Oxodaunomycinone	–OH	–H	=O
Products:	R_4	R_5	R_6
Aklavinone	–H	$–COOCH_3$	–H
ϵ-Rhodomycinone	–OH	$–COOCH_3$	–H
Daunomycinone	–OH	–H	=O

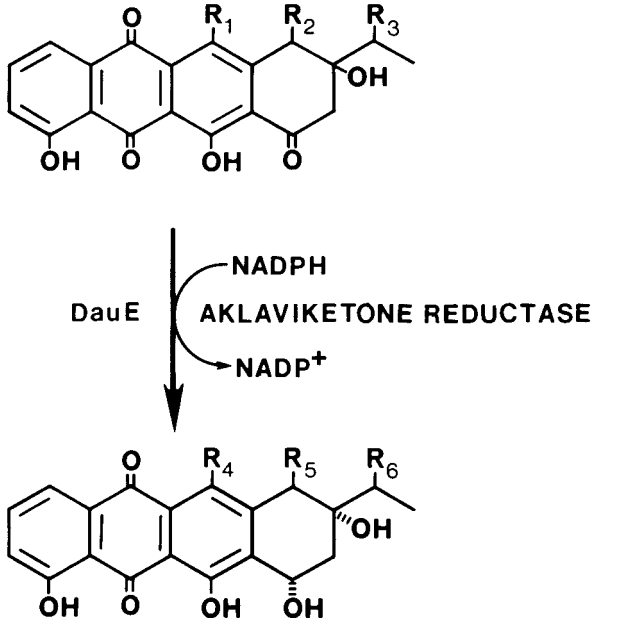

FIG. 1. NADPH-dependent conversion of aklaviketone, maggiemycin, and 7-oxodaunomycinone to aklavinone, ε-rhodomycinone, and daunomycinone, respectively, catalyzed by aklaviketone reductase. References 3 and 6 detail the proposed pathway for aklaviketone metabolism in *Streptomyces* sp. strain C5.

carrier protein (ACP) (*dauA-orfG*) (Table 1), read right to left, as depicted in Fig. 2, while the other four genes read divergently from these, left to right, in an apparent operon.

The deduced gene product of *orf1* is most closely related to *Streptomyces antibioticus* Orf4, a putative oxidoreductase of unknown function (Table 2), and also is related to several characterized polyketide ketoreductases from various streptomycetes (Fig. 3). Other than the polyketide reductase function encoded by *dauB* (32) and aklaviketone reductase, encoded by *dauE* (this work), the only remaining known ketoreductase-like function in daunomycin biosynthesis is the reduction of the C-13 keto group (9); whether *orf1* encodes this function is currently unknown.

The deduced product of *dauA-orfG*, a negatively charged protein with a predicted M_r of 9,437 and a highly conserved 4-phosphopantetheine binding site (GLDSLAV) typical of ACPs, shares 43% amino acid sequence identity with *Saccharopolyspora hirsuta* Orf6 (Table 2), which encodes an ACP putatively involved in the biosynthesis of a polyketide (20). In the more than 25 kbp of DNA within the *Streptomyces* sp. strain C5 daunomycin biosynthesis gene cluster that we have sequenced (9–11, 32), this is the only gene encoding an apparent ACP, suggesting that DauA-OrfG functions as the daunomycin biosynthesis ACP. As previously mentioned (32), the gene encoding the apparent daunomycin ACP, *dauA-orfg*, is located at least 6 kbp from the genes encoding daunomycin polyketide synthase ketoacyl synthase and the chain length factor. This gene organization is not typical of other streptomycete type II polyketide synthase genes, in which the ACP gene usually is located immediately downstream of the ketoacyl synthase and chain length factor genes (18).

The first gene reading left to right, *orf2*, encodes a small protein (deduced M_r, 16,758) of unknown function that has 45, 35, and 34% amino acid sequence identities with the *Streptomyces roseofulvus* frenolicin polyketide synthase gene cluster *orfX* product (5) (Table 2), the product of the *Streptomyces coelicolor* actinorhodin biosynthesis gene *actVI-orfA* (14), and the *Saccharopolyspora hirsuta* polyketide biosynthesis gene cluster *orfX* product (20), respectively. All of these genes except *actVI-orfA* are clustered with polyketide biosynthesis genes (5, 14, 20). Nevertheless, the functions of all of these genes and their products are unknown at this time.

The third *orf* reading left to right in the sequence shown, *dauH*, encodes an apparent glycosyltransferase that has 41%

TABLE 1. Bacterial plasmids used and constructed in this study

Plasmid	Relevant characteristics[a]	Source or reference
pUC19	2.686 kbp; Amp^r, *E. coli* plasmid	J. N. Reeve
pWHM3	7.20 kbp; shuttle vector derivative of pIJ486 and pUC19; HC, Thi^r	30
pANT148	6.27 kbp; 925-bp *Sph*I fragment from pANT151 carrying *orf1* subcloned in pANT849; plasmid promoter *snpA-p* drives transcription of *orf1*	This work
pANT151	6.4 kbp[b]; 3.7-kbp *Sst*I fragment from phage P7 in pUC19	32
pANT152	10.36 kbp[b]; 7.67-kbp *Kpn*I fragment from phage P7 in pUC19	32
pANT155	12.45 kbp; 5.25-kbp *Sst*I fragment from pANT152 in pWHM3 (contains intact *dauE* gene)	This work
pANT156	11.34 kbp; 4.14-kbp *Eco*RI-*Sst*I fragment from pANT152 in pWHM3 (contains truncated *dauE* gene)	This work
pANT157	4.62 kbp; 1.93-kbp *Pvu*II-*Sst*I fragment from pANT152 cloned into *Sma*I-*Sst*I sites of pUC19	This work
pANT159	7.28 kbp; 1.94-kbp *Bam*HI-*Sst*I fragment from pANT157 cloned into pANT849 so that vector *snpA* promoter drives expression of *dauE*	This work
pANT164	9.24 kbp; pIJ486 carrying 3.02-kbp *Eco*RI-*Kpn*I insert from pANT121 (contains expressed *dauB*)	32
pANT849	5.343 kbp: expression vector derived from pANT42 (19) by removal of a 1.95-kbp *Kpn*I fragment and a 1.42-kbp *Sph*I-*Mlu*I fragment and insertion of a 42-bp polylinker into resulting *Sph*I-*Mlu*I sites; expression of the genes cloned into the polylinker is driven by SnpR-activated *snpA* promoter; HC, Thi^r	7

[a] Abbreviations: HC, high-copy-number plasmid; Thi^r, thiostrepton resistance; Amp^r, ampicillin resistance.
[b] The size of this plasmid and its insert have been revised from that reported in Ye et al. (32).

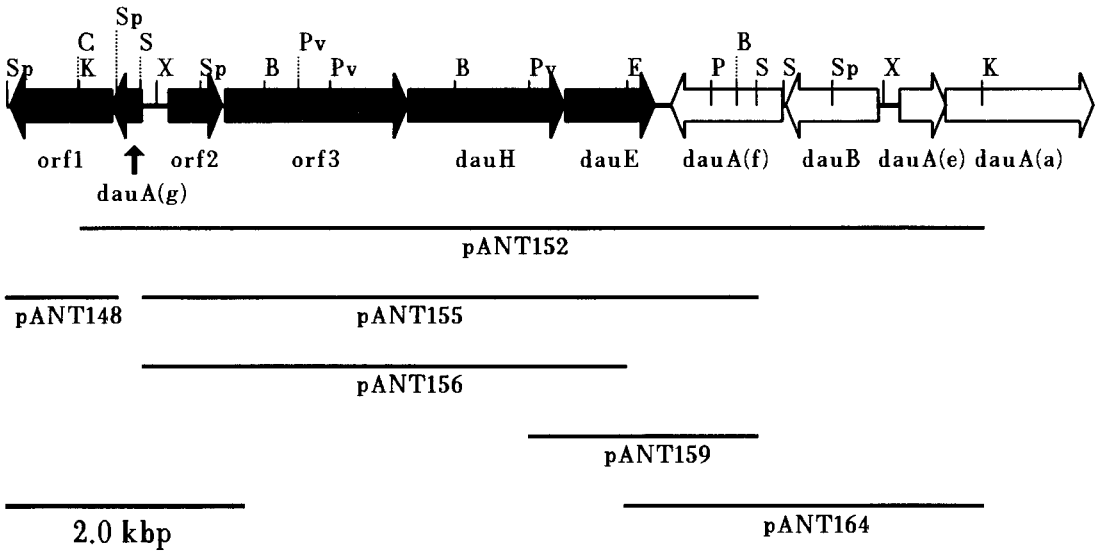

FIG. 2. Restriction map, generated by complete nucleotide sequence data, of part of the daunomycin biosynthesis gene cluster from *Streptomyces* sp. strain C5. Genes within the ca. 6.0-kbp DNA sequence from the left end at the *Sph*I site to the end of the *dauE* gene (black arrows) are described in the text. The genes on the right (white arrows) are described in reference 32. The three genes encoding ketoreductases are *orf1*, *dauE*, and *dauB*. The inserts of plasmids pANT148, pANT152, pANT155, pANT156, pANT159, and pANT164 are indicated. Abbreviations for restriction endonuclease sites: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; K, *Kpn*I; Pv, *Pvu*II; S, *Sst*I; X, *Xho*I; Sp, *Sph*I.

amino acid sequence identity with *Streptomyces peucetius* DnrS, a glycosyltransferase encoded by a gene located downstream of *dnrK* and *dnrP* in the daunomycin biosynthesis gene cluster of that organism (15) (Table 2). The function of DauH in daunomycin biosynthesis in *Streptomyces* sp. strain C5 is not known, although it could theoretically catalyze either the condensation of ε-rhodomycinone and TDP-daunosamine to form the hypothesized intermediate rhodomycin (27) or, alternatively, it might encode the enzyme that converts daunomycin to baumycin A1/A2 (23). *Streptomyces peucetius* DnrS was proposed to encode TDP-daunosamine:ε-rhodomycinone glycosyltransferase, although unequivocal evidence for its role is still lacking (24).

The final gene in the newly sequenced DNA encodes a protein, here named DauE, with a deduced M_r of 25,632 that was homologous to several streptomycete ketoreductases (Fig. 3). DauE contained 97% amino acid sequence identity with *Streptomyces peucetius* DnrH, a deduced ketoreductase of unknown function previously sequenced by Grimm et al. (15).

Complementation of *dauE* strains. Aklaviketone reductase activity (Fig. 1) would theoretically be catalyzed by the product of a gene belonging to a ketoreductase family. Since the sequence upstream of the daunomycin polyketide synthase gene cluster contained genes encoding two new ketoreductases, we analyzed them (encoded by *orf1* and *dauE*), as well as *dauB*, previously shown to encode anthracycline polyketide reductase activity (32), for their ability to complement *dauE* mutants and to catalyze aklaviketone reductase activity. Subclones of all three ketoreductase genes (Table 1; Fig. 2) were constructed for transformation of *dauE* mutants and for heterologous expression in *Streptomyces lividans* TK24.

Plasmids pANT155 and pANT159, both containing an intact *dauE* gene, complemented the maggiemycin-producing *Streptomyces* sp. strain C5 *dauE* mutants SC5-24, SC5-110, and SC5-111 (2, 3) to restore daunomycin biosynthesis, as determined by thin-layer chromatography and high-performance liquid chromatography (HPLC) analyses of the products, by methods previously described (32).

Plasmid pANT156, containing a truncated form of *dauE*, did not restore daunomycin biosynthesis to *dauE* mutants SC5-24, SC5-110, and SC5-111. Similarly, pANT148, containing an intact copy of *orf1* under control of the *snpA-p* promoter (7), and pANT164, containing *dauB* (32), did not complement any of the *dauE* strains.

In vivo bioconversions. Plasmids containing each of the three ketoreductase genes were introduced into protoplasts of *Streptomyces lividans* TK24 by transformation (17). After the transformants were grown in liquid YEME plus 10 μg of thiostrepton per ml at 30°C (rotary shaking at 250 rpm) for 48 h,

TABLE 2. Characteristics of predicted gene products

Open reading frame product	Length (no. of residues)	Predicted M_r	Probable function[a]	Closest homolog (% identity)	Reference
Orf1	287	30,799	Ketoreductase	*Streptomyces antibioticus* Orf4 (30%)	33
DauA-OrfG	84	9,437	Acyl carrier protein	*Saccharopolyspora hirsuta* ACP (43%)	20
Orf2	151	16,758	Unknown	*Saccharopolyspora hirsuta* OrfX (45%)	20
Orf3	516	56,316	Unknown	None	
DauH	442	47,977	Glycosyltransferase	*Streptomyces peucetius* DnrS (41%)	24
DauE	251	25,632	Aklaviketone 7-reductase	*Streptomyces peucetius* DnrH (97%)	15

[a] All gene products described are expected to be involved in daunomycin biosynthesis.

```
DauE                MENTQRSV IVTGGGSGIG RAVA.RAFAA RGDRVLV.VG RTAGPLAETV DGH.KEA... .HTLAVDITD PAAPQAVVRE VRERLGGVVD
DnrH                MENTQRSV IVTGGGSGIG RAVA.RAFAA RGDRVLV.VG RTAGPLAETV DGH.KDA... .HTLAVDITD PAAPEAVVRE VRERLGGVVD
Orf1     MTVPTPRHGT PHGGLPGRTV LITGATSGIG RAAAL.AIAR QGARVVL.VG RDPERLRTVT NEVARTAGPA PDAFRADFAE LRQVRELGER LRDRY.PRID
DauB               MSDAADRVA LVTGGTSGIG LAVGRRKLAL DGTRVFL.CA RDESAVTGTV KEL.QASGLE VEGTSCDVRS TDAVDRLVRT ARNPLRA.ID
AknA               MPPAAERVA IVTGATSGIG LAVA.RSLAE GGARVFV.CA RDGDRVAHTV KEL.REAGHD VDGASCDVRD TARVRAFVQE ARDRFGP.VD
ActIII             MATQDSEVA LVTGATSGIG LEIARR.LGK EGLRVFV.CA RGEEGLRTTL KEL.REAGVE ADGRTCDVRS VPEIEALVAA VVERYGP.VD
Gra-5    MTTATATATA TPGTAAKPVA LVTGATSGIG LAIARR.LAA LGARTFL.CA RDEERLAQTV KEL.RGEGFD VDGTVCDVAD PAQIRAYVAA AVQRYGT.VD
Gra-6            M ATDAPEAPVA LVTGSSSGIG QTVAQR.LAA EGYRVVVNSA RSVEDGEKTA AAL.PDALY. ...VRADVSE EADARRLVDT AVEHYGR.LD
Cons.              mgtaa.rva lvTGatSGIG lavarr.laa eGaRvfv.ca Rdeerlattv kel.reag.. vdgt.cDvrd .a.vralv.. arerygp.vD

DauE     VLVNNAATAV FGHLGELDR. ..TAVEAQVA TNLVAPVLLT QALLDP...L ETASGLVVNI GSAGALG.RR AWPGNAVYGA AKAGLD.... ........LL
DnrH     VLVNNAATAA FGHLGELHR. ..TAVEAQVA TNLVAPVLLT QALLGP...L ETASGLVVNI GSAGALG.RR AWPGNAVYGA AKAGLD.... ........LL
Orf1     VMAGNAGGMF WSRTTTQDGF EATLQVNHLA GFLLARLLRE RLAGGRLILT SSDAYTQGRI DPDDLNGDRH RYSAGQAYGT SKQANIMTAT EAARRWPDVL
DauB     IVVNNAGRGG GGVTAQITD. ..DLWSDVVD TNLGGAFRVT RAVLTGGGMQ EHGWGRIINI ASTG..G.KQ GVALGAPYSA SKSGLI.... ........GF
AknA     VLVNNAGRSG GGHTAQIPD. ..ELWLDVIE TNLNSVFRMT REVLTTGGML ERGAGRIVNI ASTG..G.KQ GVALGAPCSA SKHGVV.... ........GF
ActIII   VLVNNAGRPG GGATAELAD. ..ELWLDVVE TNLTGVFRVT KQVLKAGGML ERGTGRIVNI ASTG..G.KQ GVVHAAPYSA SKHGVV.... ........GF
Gra-5    ILVNNAGRSG GGATAEIAD. ..ELWLDVIT TNLTSVFLMT KEVLNAGGML AKKRGRIINI ASTG..G.KQ GVVHAVPYSA SKHGVV.... ........GL
Gra-6    VLVNNAGRTR AIPHADLAAA TPEVWREILG LNVIGTWQTT VAAMPH..LA RSGNGSVVNV SSIA..G.SR PAGSSIPYAV SNGGHR.... ........AQ
Cons.    vlvnNAgrsg gg.tae.ad. ..elwldvv. tnltgvf..t ravltaggml e.gagrivni astg..G.kq gv..gapysa skhgvv.... ........g.

DauE     TRSWAVELGP RGIRVIGVAP GVIET..GAG VR...AGMSQ EAYDGFLEAM GQRV.....P LGRVGRPEDV AWWVVRLADP EAAYASGAVL AVDGGLSVT*
DnrH     TRSWAVELGP RGIRVVGVAP GVIGT..GAG VR...AGMSQ EAYDGFLEAM GQRV.....P LGRVGRPEDV AWWVVRLADP EAAYASGAVL AVDGGLSVT*
Orf1     TVSYHPGEVR TRIGRGTVAS TYFRFNPFLR SAAKGADTLV WLAAAPAEEL TTGGYYSDRR LSPVSGPTAD AGLAAKLWEA SAAAVGDTAR *
DauB     TKAVALELAR TGITVNAVCP GYVETPMAQG VRQRYAAFWG ITEDDVLEKF RAKI.....P LGRYSTSDEV AGMVHYLVSD SADSITAQAI NVCGGLGSY*
AknA     TKALGLELAK TGITVNAVCP GYVETPMAER VRQGYAGAWD ITEDEVLERF EAKI.....P LGRYSTPDEV AGLVGYLLSS TAASITAQAM NVCGALGNY*
ActIII   TKALGLELAR TGITVNAVCP GFVETPMAAS VREHYSDIWE VSTEEAFDRI TARV.....P IGRYVQPSEV AEMVAYLIGP GAAAVTAQAL NVCGGLGNY*
Gra-5    TKALGLELAR TGITVNAVCP GFVETPMAER VREHYAGIWQ VSEEETFDRI TNRV.....P LGRYVETREV AAMVEYLVAD DAAAVTAQAL NVCGGLGNY*
Gra-6    TRLLANTVGP .AVRVNAVAP GLIETP.... .WTQNSDFFA ....PIAEHV RQTT.....P LRRTGRPEDV AEAVLGLV.. RATYTTGQVL LVDGGAHLL*
Cons.    Tkal.lelar tgitvnaVcp gyvetpma.r vr.hya..wq ..edevler. tarv.....p lgrys.p.ev AgmvayLvs. sAaavtaqal nvcgglgny
```

FIG. 3. LINEUP analysis of the PILEUP comparison (8) of the deduced amino acid sequences of *Streptomyces* sp. strain C5 DauE with other antibiotic biosynthesis-related ketoreductases: *Streptomyces peucetius* ATCC 29050 DnrH (15), *Streptomyces* sp. strain C5 Orf1 (this work), *Streptomyces* sp. strain C5 DauB (32), *Streptomyces galilaeus* ATCC 31133 AknA (29), *Streptomyces coelicolor* ActIII (16), and *Streptomyces violaceoruber* Gra-Orf5 and Gra-Orf6 (26). Dots indicate gaps generated by PILEUP, and the consensus sequence shown was determined by LINEUP. Capital letters in the consensus strand indicate identical residues in that position, and small letters indicate at least four of eight proteins containing identical residues in the position. The parameters used were a gap weight of 3.0 and a gap length weight of 0.1. Cons., consensus sequence.

1.0 μmol (final concentration) of aklaviketone was added to the culture broths, and the cultures were incubated for an additional 48 h to test their ability to convert aklaviketone to aklavinone. *Streptomyces lividans* TK24(pANT159) converted 100% of the added aklaviketone to aklavinone, whereas cultures of *Streptomyces lividans*(pANT148), containing *orf1*, *Streptomyces lividans*(pANT164), containing *dauB*, and *Streptomyces lividans* TK24(pANT849), the control culture, converted less than 10% of the aklaviketone to aklavinone.

In vitro enzyme activities. Desalted mycelial extracts were prepared, by methods previously described (6), from 48-h recombinant *Streptomyces lividans* TK24 cultures grown in YEME plus 10 μg of thiostrepton per ml to determine in vitro aklaviketone reductase activities. Aklaviketone reductase assays included, in a 500-μl volume, desalted cell extract, 0.5 mg of protein; aklaviketone (or another 7-oxo-anthracyclinone), 10 nmol; NAD(P)H, 20 nmol; and sodium phosphate buffer (pH 7.5), 50 μmol. The assays were incubated for periods of 5 to 60 min at 37°C, and the products were then extracted with chloroform-methanol (9:1). The organic phase was separated, dried, reconstituted in 10 μl of chloroform–90 μl of methanol, and separated and quantified by HPLC with a Waters C_{18} μBondapak reverse-phase column and a mobile phase of methanol-water (65:35) brought to a pH of 2.5 with concentrated acetic acid. A Waters model 600E multisolvent delivery pump and controller and model U6K 0- to 2.0-ml manual injector were used, and anthracyclines were detected on-line at 254 nm with a Waters model 486 tunable absorbance detector. The data were analyzed on-line with Baseline 815 software and a 386SX IBM-compatible computer. The elution times of bioconversion products were compared with those of authentic standards run in parallel. Retention times for the anthracyclinone standards were 9.3 min for aklaviketone, 11.4 min for aklavinone, 10.4 min for maggiemycin, 15.1 min for ε-rhodomycinone, 6.9 min for 7-oxodaunomycinone, and 10.1 min for daunomycinone.

Table 3 shows that desalted extracts of *Streptomyces lividans* TK24(pANT159), containing recombinant DauE, catalyzed the NADPH-specific conversion of aklaviketone to aklavinone. Connors et al. (6) also found that aklaviketone activity in *Streptomyces* sp. strain C5 was NADPH specific. The specific activity for aklaviketone reduction to aklavinone by *Streptomyces lividans* TK24(pANT159) extracts was 62 pmol/min/mg of protein, and the reaction was determined to be linear for up to 60 min under the conditions described. *Streptomyces lividans* TK24(pANT159) extracts also catalyzed the NADPH-specific reduction of maggiemycin to ε-rhodomycinone at a rate approximating that of the aklaviketone reduction, indicating that maggiemycin also is a substrate for this enzyme. This result was unexpected, since extracts of *Streptomyces* sp. strain C5 previously were not found to convert maggiemycin to ε-rhodomycinone (6). Similarly, *Streptomyces lividans* TK24(pANT159) extracts also catalyzed the NADPH-specific reduction of 7-oxodaunomycinone to daunomycinone, but at a rate about

TABLE 3. In vitro reduction of aklaviketone to aklavinone by recombinant *Streptomyces lividans* TK24 extracts[a]

Plasmid	Pertinent ketoreductase	Coenzyme added	% conversion
None	None	NADPH	2.0
pANT148	Orf1	NADPH	2.9
pANT159	DauE	NADPH	100
pANT159	DauE	NADH	7.0
pANT159	DauE	None	11.0
pANT164	DauB	NADPH	1.8
pANT849	None	NADPH	2.5

[a] The results reported are from duplicate assays carried out for 60 min and analyzed by HPLC as described in the text. The substrate concentrations used were 20 μM for aklaviketone and 40 μM for NAD(P)H. Similar results also were obtained in five independent replications of this experiment with modifications in time and substrate concentration.

half those of the other two reactions reported above (11). In control experiments, extracts prepared from cultures of *S. lividans* TK24(pANT849), *Streptomyces lividans* TK24(pANT148), or *Streptomyces lividans* TK24(pANT164) incubated with aklaviketone and NADPH catalyzed only background levels of aklaviketone reductase activity (Table 3).

Nucleotide sequence accession number. The DNA sequence of the genes described in this paper has been deposited in GenBank under the accession no. U43704.

We are extremely grateful to Klaus Eckardt and his colleagues of the Academy of Sciences, Jena, Germany, for authentic samples of aklaviketone. We also thank C. Richard Hutchinson for the shuttle vector pWHM3.

This work was supported by the National Science Foundation (grant MCB-94-05730) and in part by NIH grant 43345.

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Bartel, P. L.** 1989. Analysis of natural and hybrid antibiotic formation in anthracycline-producing streptomycetes. Ph.D. dissertation, The Ohio State University, Columbus.
3. **Bartel, P. L., N. C. Connors, and W. R. Strohl.** 1990. Biosynthesis of anthracyclines: analysis of mutants of *Streptomyces* sp. strain C5 blocked in daunomycin biosynthesis. J. Gen. Microbiol. **136:**1877–1886.
4. **Bibb, M. J., P. R. Findlay, and M. W. Johnson.** 1984. The relationship between base composition and codon usage in bacterial genes and its use for the simple and reliable identification of protein-coding sequences. Gene **30:**157–166.
5. **Bibb, M. J., D. H. Sherman, S. Omura, and D. A. Hopwood.** 1994. Cloning, sequencing and deduced functions of a cluster of *Streptomyces* genes probably encoding biosynthesis of the polyketide antibiotic frenolicin. Gene **142:**31–39.
6. **Connors, N. C., P. L. Bartel, and W. R. Strohl.** 1990. Biosynthesis of anthracyclines: enzymic conversion of aklanonic acid to aklavinone and ε-rhodomycinone by anthracycline-producing streptomycetes. J. Gen. Microbiol. **136:**1887–1894.
7. **DeSanti, C., J. S. Lampel, K. A. Lampel, and W. R. Strohl.** Unpublished data.
8. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
9. **Dickens, M. L., and W. R. Strohl.** 1996. Isolation and characterization of a gene from *Streptomyces* sp. strain C5 that confers the ability to convert daunomycin to doxorubicin on *Streptomyces lividans* TK24. J. Bacteriol. **178:**3389–3395.
10. **Dickens, M. L., J. Ye, and W. R. Strohl.** 1995. Analysis of clustered genes encoding both early and late steps in daunomycin biosynthesis by *Streptomyces* sp. strain C5. J. Bacteriol. **177:**536–543.
11. **Dickens, M. L., J. Ye, and W. R. Strohl.** Unpublished data.
12. **Eckardt, K., G. Schumann, D. Tresselt, and W. Ihn.** 1988. Biosynthesis of anthracyclinones: isolation of a new early cyclization product aklaviketone. J. Antibiot. **41:**788–793.
13. **Eckardt, K., and C. Wagner.** 1988. Biosynthesis of anthracyclinones. J. Basic Microbiol. **28:**137–144.
14. **Fernández-Moreno, M. A., E. Martínez, J. L. Caballero, K. Ichinose, D. A. Hopwood, and F. Malpartida.** 1994. DNA sequence and functions of the *actVI* region of the actinorhodin biosynthetic gene cluster of *Streptomyces coelicolor* A3(2). J. Biol. Chem. **269:**24854–24863.
15. **Grimm, A., K. Madduri, A. Ali, and C. R. Hutchinson.** 1994. Characterization of the *Streptomyces peucetius* ATCC 29050 genes encoding doxorubicin polyketide synthase. Gene **151:**1–10.
16. **Hallam, S. E., F. Malpartida, and D. A. Hopwood.** 1988. Nucleotide sequence, transcription and deduced function of a gene involved in polyketide antibiotic synthesis in *Streptomyces coelicolor*. Gene **74:**305–320.
17. **Hopwood, D. A., M. J. Bibb, K. F. Chater, T. Kieser, C. J. Bruton, H. M. Kieser, D. J. Lydiate, C. P. Smith, J. M. Ward, and H. Schrempf.** 1985. Genetic manipulation of *Streptomyces*: a laboratory manual. The John Innes Foundation, Norwich, United Kingdom.
18. **Hopwood, D. A., and D. H. Sherman.** 1990. Molecular genetics of polyketides and its comparison to fatty acid biosynthesis. Annu. Rev. Genet. **24:**37–66.
19. **Lampel, J. S., J. S. Aphale, K. A. Lampel, and W. R. Strohl.** 1992. Cloning and sequencing of a gene encoding a novel extracellular neutral proteinase from *Streptomyces* sp. strain C5 and expression of the gene in *Streptomyces lividans* 1326. J. Bacteriol. **174:**2797–2808.
20. **Le Gouill, C., D. Desmarais, and C. V. Dery.** 1993. *Saccharopolyspora hirsuta* 367 encodes clustered genes similar to ketoacyl synthase, ketoacyl reductase, acyl carrier protein, and biotin carboxyl carrier protein. Mol. Gen. Genet. **240:**146–150.
21. **Madduri, K., and C. R. Hutchinson.** 1995. Functional characterization and transcriptional analysis of a gene cluster governing early and late steps in daunorubicin biosynthesis in *Streptomyces peucetius*. J. Bacteriol. **177:**3879–3884.
22. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **McGuire, J., M. C. Thomas, R. C. Pandey, M. Toussaint, and R. J. White.** 1981. Biosynthesis of daunorubicin glycosides: analysis with blocked mutants, p. 117–122. *In* M. Moo-Young (ed.), Advances in biotechnology, vol. 3. Permagon Press, New York.
24. **Otten, S. L., X. Liu, J. Ferguson, and C. R. Hutchinson.** 1995. Cloning and characterization of the *Streptomyces peucetius dnrQS* gene encoding a daunosamine biosynthesis enzyme and a glycosyl transferase involved in daunorubicin biosynthesis. J. Bacteriol. **177:**6688–6692.
25. **Pandey, R. C., M. W. Toussaint, J. C. McGuire, and M. C. Thomas.** 1989. Maggiemycin and anhydromaggiemycin: two novel anthracyclinone antitumor antibiotics. J. Antibiot. **42:**1567–1577.
26. **Sherman, D. H., F. Malpartida, M. J. Bibb, H. M. Kieser, M. J. Bibb, and D. A. Hopwood.** 1989. Structure and deduced function of the granaticin-producing polyketide synthase gene cluster of *Streptomyces violaceoruber* Tü22. EMBO J. **9:**2717–2725.
27. **Strohl, W. R., P. L. Bartel, N. C. Connors, C.-B. Zhu, D. C. Dosch, J. M. Beale, Jr., H. G. Floss, K. Stutzman-Engwall, S. L. Otten, and C. R. Hutchinson.** 1989. Biosynthesis of natural and hybrid polyketides by anthracycline-producing streptomycetes, p. 68–84. *In* C. L. Hershberger, S. W. Queener, and G. Hegeman (ed.), Genetics and molecular biology of industrial microorganisms. American Society for Microbiology, Washington, D.C.
28. **Strohl, W. R., and N. C. Connors.** 1992. Significance of anthraquinone formation resulting from the cloning of actinorhodin genes in heterologous streptomycetes. Mol. Microbiol. **6:**147–152.
29. **Tsukamato, N., I. Fujii, Y. Ebizuka, and U. Sankawa.** 1994. Nucleotide sequence of the *aknA* region of the aklavinone biosynthetic gene cluster of *Streptomyces galilaeus*. J. Bacteriol. **176:**2473–2475.
30. **Vara, J., M. Lewandowska-Skarbek, Y.-G. Wang, S. Donadio, and C. R. Hutchinson.** 1989. Cloning of genes governing the deoxysugar portion of the erythromycin biosynthesis pathway in *Saccharopolyspora erythraea* (*Streptomyces erythreus*). J. Bacteriol. **171:**5872–5881.
31. **Wright, F., and M. J. Bibb.** 1992. Codon usage in the G+C-rich *Streptomyces* genome. Gene **113:**55–65.
32. **Ye, J., M. L. Dickens, R. Plater, Y. Li, J. Lawrence, and W. R. Strohl.** 1994. Isolation and sequence analysis of the polyketide synthase genes from the daunomycin-producing *Streptomyces* sp. strain C5. J. Bacteriol. **176:**6270–6280.
33. **Yu, T.-W., and C. W. Chen.** 1993. The unstable *melC* operon of *Streptomyces antibioticus* is codeleted with a Tn*4811*-homologous locus. J. Bacteriol. **175:** 1847–1852.