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Incorrectly folded aromatic polyketides from polyketide reductase deficient mutants

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Abstract: Compounds produced by the polyketide ketoreductase deficient *Streptomyces* mutants HO61 and P67 are described. The structures of the compounds indicate that ketoreductase activity is required for correct condensation of the polyketide chain in the biosynthesis of aromatic polyketides.

Polyketides are a complex and pharmaceutically important class of compounds produced as secondary metabolites by various soil bacteria. Structural diversity of the polyketides arises from the multifunctional enzyme complex, polyketidesynthase (PKS). Functions of the different enzymes in PKS can be investigated through the secondary metabolites, since they result from the enzymatic activity encoded by the genes. In principle the order of biosynthetic steps could be constructed by elucidating the structures of the biosynthetic intermediates. However, most of the biosynthetic intermediates are reactive in nature and conclusions have to be drawn via shunt products. Strains used in biosynthetic studies can generally be classified as either genetically engineered or mutated. In the former, a desired combination of genes are expressed in a nonproducing strain.[1] A complication is the need to temper the enzymatic activities of the hosts, which are capable of increasing the structural variation of the products via minor modifications (e.g. oxidation, reduction, methylation).[2] As a result, each main compound is often accompanied by several slightly modified, minor products, reflecting not only the enzymatic activity of the desired genes, but also those of the host. A larger number of products increases the possibility for misinterpretation. On the other hand, mutants deficient in a single enzyme avoid or diminish the number of minor shunt products, thus facilitating a more straightforward interpretation.

Mutants P67 and HO61 were derived from *Streptomyces peucetius* ATCC 27952 and *S. galilaeus* ATCC 31365, respectively, by random chemical mutagenesis.[3] Fermentations were conducted on a 10 l scale using a medium composed of glucose, starch, Pharmamedia (Traders protein), yeast extract, $CaCO_3$, NaCl, $MgSO_4$, and KH_2PO_4 in tap water. After 130 hours the culture was exhaustively extracted (CH_2Cl_2:MeOH 3:1) at pH 3. One major product, **1** (60%), and several minor products (≤5%) were detected by HPLC analysis of the crude liquid culture extract from HO61. HPLC analysis of the extract from P67 showed that it was composed of two major products, **1** and **2** in a 1:3 ratio, and several minor components. Isolation of the main product from each culture extract by polyamide 11 column flash chromatography (MeOH:AcOH 99:1) and semipreparative HPLC (RP-18ec) (CH_3CN:H_2O:CH_3CO_2H 40:59:1) yielded 110 mg of **1** (98% pure) and 20 mg of **2** (95% pure). For both **1** and **2** three substructures were readily identified from the ^1H NMR spectrum (Table 1): an ethyl group, a 2-

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hydroxy-4-pyrone ring, and an anthraquinone ring. The four hydroxyl proton signals, confirmed by saturation transfer upon irradiation of the water signal, resonated between 12.7 and 11.2 ppm. The two low-field hydroxyl signals of **1** were sharp and concentration independent, with respect to the chemical shift and peak shape, indicating their involvement in intramolecular hydrogen-bonding. Of the 21 carbon signals in the ^{13}C NMR spectrum (Table 1) 18 resonated downfield of 100 ppm, one at 90 ppm, and the two ethyl group signals at *ca.* 26 and 15 ppm. Carbons were unambiguously assigned using a standard combination of DEPT, HSQC, and HMBC experiments. Since the structural identification of the products relied so heavily on the HMBC correlations, they are explicitly shown in Figure 1. The EIMS of both **1** and **2** gave the correct molecular mass (394) for each and expected degradation patterns consistent with the structures.

Table 1. ^{1}H and ^{13}C NMR spectroscopic data (at 400 and 100 MHz, respectively) of **1** and **2** in d_6-DMSO.

Site	^{1}H/ppm, mult., J_{HH}/Hz, area)	^{13}C/ppm (multiplicity)	^{1}H/ppm, mult., J_{HH}/Hz, area)	^{13}C/ppm (multiplicity)
	1	**1**	**2**	**2**
1	-	164.5(s)	-	163.9(s)
1-OH	11.70, brs, 1H	-	exchange broadened	-
2	5,47, d, 2.0, 1H	89.9(d)	5.47, d, 1.8, 1H	89.6(d)
3	-	169.9(s)		170.2(s)
4	6.22, d, 2.0, 1H	105.1(d)	6.6.28, 1.8, 1H	104.6(d)
5	-	156.1(s)	-	157.7(s)
6	-	127.7(s)	-	127.2(s)
7	-	159.5(s)	-	160.5(s)
7-OH	12.67, s, 1H	-	exchange broadened	-
8	-	108.1(s)	7.73, s, 1H	112.6(d)
9	-	189.3(s)	-	137.5(s)
10	-	113.8(s)	-	182.1(s)
11	-	164.4(s)	-	133.9(s)
11-OH	12.01, s, 1H	-	-	-
12	6.61, d, 2.4, 1H	109.0(d)	7.09, d, 1.8, 1H	107.3(d)
13	-	165.8(s)	-	164.3(s)
13-OH	11.21, brs, 1H	-	exchange broadened	-
14	7.21, d, 2.4, 1H	109.1(d)	6.64, d, 1.8, 1H	108.5(d)
15	-	133.5(s)	-	164.6(s)
15-OH	-	-	exchange broadened	-
16	-	180.9(s)	-	110.3(s)
17	-	135.0(s)	-	187.5(s)
18	7.67, s, 1H	119.0(d)	-	123.5(s)
19	-	152.4(s)	-	149.7(s)
20	2.71, q, 7.5, 2H	26.6(t)	3.04, q, 7.4, 2H	25.1(t)
21	1.25, t, 7.5, 3H	14.7(q)	1.14, t, 7.4, 3H	15.1(q)

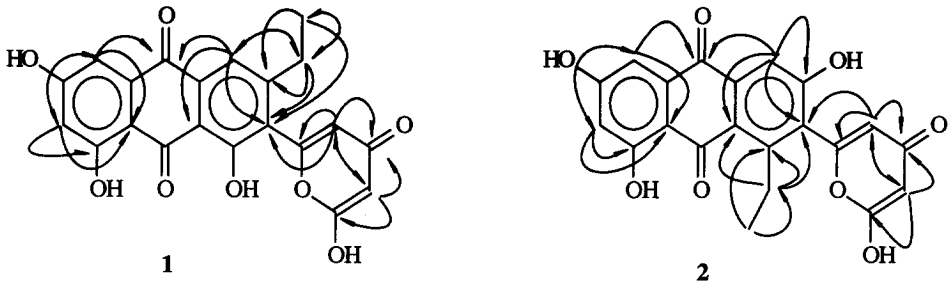

Figure 1. Observed HMBC correlations for **1** and **2**.

The importance of strains HO61 and P67 lies in the fact that only one enzymatic activity is destroyed in the organism leaving all other functions fully operational. Both strains are blocked at an early stage of polyketide biosynthesis; this was deduced by introducing a plasmid containing the gene encoding nogalamycin polyketide ketoreductase into HO61.[4] Positive complementation was indicated by the resultant accumulation of aklavinone **3**, a secondary metabolite of the parent strain, and the disappearance of both **1** and **2** from the culture broth.

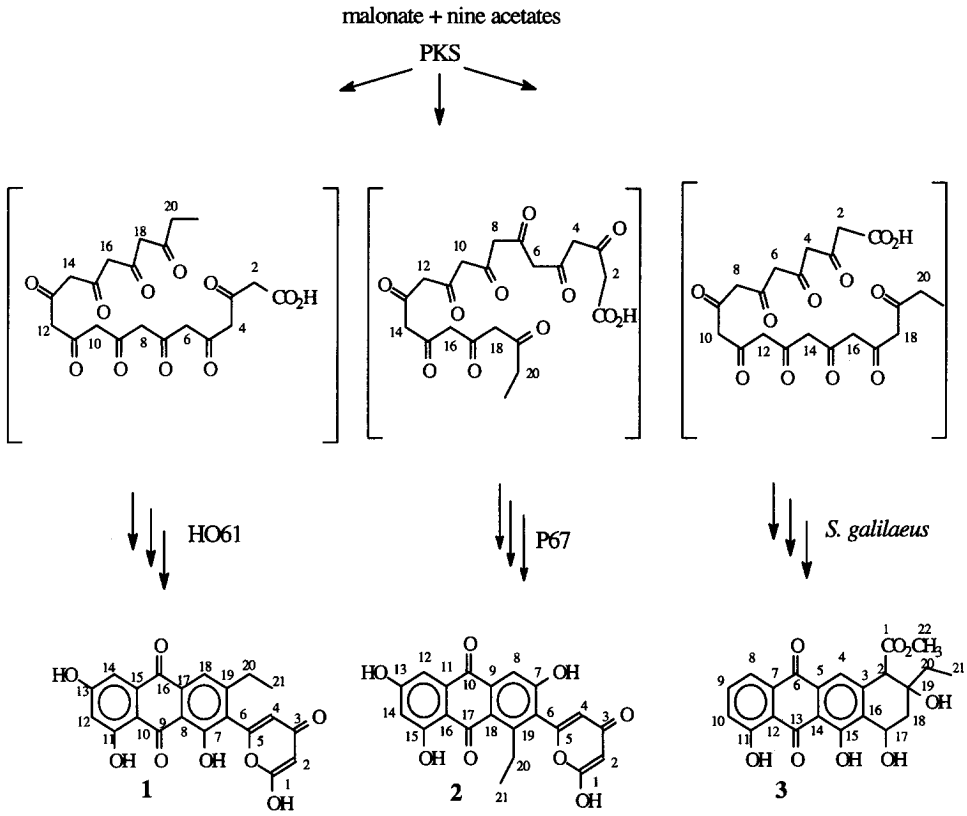

Figure 2. Structures of nascent polyketides leading to isolated compounds **1**, **2**, and **3**.

Ketoreductase (KR) is the first enzyme to affect the nascent polyketide chain and has therefore a pronounced role in dictating the structure of the final product. The primary function of KR is to reduce the carbonyl group at C-9 of the nascent polyketide. However, reduction also orientates the polyketide chain favourably for an aldol condensation to occur between C-7 and C-12 and thereby facilitating the cyclisation of the first ring. The structure of **1** though, requires that aldol condensation occurs between C-10 and C-15 for the formation of the analogous ring. This difference from the condensation of **3** suggests that KR directs the folding of the polyketide chain (Figure 2), and that KR is indeed necessary for the condensation of the polyketide chain to occur correctly. The appearance of **1** and **2** thus indicate flexibility with regard to the correct condensation pattern of polyketide if KR is not functional. Contrary to earlier results, KR does not appear to be responsible for determining the chain length of the polyketide, as this is similar in compounds produced by both wild-type and mutant strains.[5]

These results from strains HO61 and P67 indicate that earlier conclusions concerning the biosynthesis of polyketides are likely to be oversimplified. The functions of individual enzymes within PKS are influenced by other enzymes and by the environment, leading to variation of metabolite output.

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